Society of Toxicology

41st Annual Meeting & ToxExpo™

An Official Journal of the Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES
Formerly Fundamental and Applied Toxicology

Abstracts of the 41st Annual Meeting

The Toxicologist

Oxford University Press

Volume 66, Number 1-5, March 2002
Preface

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

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

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

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

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

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Society of Toxicology
1767 Business Center Drive, Suite 302
Reston, VA 20190-5332

http://www.toxicology.org

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

J. I. Everett, CIIT Center for Health Research, Research Triangle Park, NC.

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

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


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

ALTERATIONS IN GENE EXPRESSION AS A MECHANISM OF TOXICANT ACTION.

R. N. Hines', Q. Ma', G. K. Andrews' and P. E. Mirkov', 'Medical College of Wisconsin, Milwaukee, WI; 'CDC/NIOSH, Morgantown, WV; 'University of Kansas Medical Center, Kansas City, KS and 'University of Washington, Seattle, WA.

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

INTEGRATING TOXICOLOGIC PATHOLOGY INTO COMPOUND EVALUATION AND RISK ASSESSMENT.

D. C. Wolf', J. E. Harhabay', R. T. Miller' and J. I. Everett', 'USEPA, Durham, NC; 'Experimental Pathology Laboratory, Research Triangle Park, NC; 'GhassanSmithKline, Research Triangle Park, NC and 'CIIT Center for Health Research, Research Triangle Park, NC.

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

BASIC PRINCIPLES AND PROTOCOLS IN MOLECULAR TOXICOLOGY.

W. B. Martens', J. W. Davis', 'E. M. K. Walker', 'P. J. Yagan Huang', 'M. S. Denison' and G. B. Marcus', 'Pharmacia, Kalamazoo, MI; 'Scribner's Porch Institute, Lafayette, NJ; 'University of New Mexico, Albuquerque, NM; 'Pennsylvania State University, Pennsylvania State University, PA and 'University of California-Davis, Davis, CA.

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

TWO-STEPPING THROUGH TOXICOGENOMICS: A BASIC PRIMER.

M. J. Cunningham', T. Zacharewski', R. Somogyi' and B. A. Merrick', 'Molecular Mining Corporation, Kingston, ON, Canada; 'Michigan State University, East Lansing, MI and 'NIEHS, Research Triangle Park, NC.

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


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

8 INCORPORATION OF PHARMACOKINETIC AND PHARMACODYNAMIC DATA INTO RISK ASSESSMENTS.


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

9 TOXICOLOGY OF NATURALLY OCCURRING TOXINS - DON’T MESS WITH MOTHER NATURE.

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

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

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

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

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

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

B. S. Shane†, M. Poizner†, L. Marner†, R. Tice and B. Finette. 1. Integrated Laboratory Systems, Research Triangle Park, NC; 2. NIH, Bethesda, MD; 3. Vanderbilt University Medical Center, Nashville, TN; 4. Integrated Laboratory Systems, Durham, NC and 5. University of Vermont, Burlington, VT.

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

2 SOT 2002 ANNUAL MEETING
12 TOXICITY PROFILING OF GENES AND PROTEINS BY TOXICOLOGISTS: ADVANCED TOPICS IN TOXICOGENOMICS.


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

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


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

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


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

15 IMAGING THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE IN TOXIC INJURY.

D. A. Fox* and J. I. Lemasters. *University of Houston, Houston, TX and **University of North Carolina, Chapel Hill, NC.

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

16 PATHOPHYSIOLOGY OF THE MITOCHONDRIAL PERMEABILITY TRANSITION.

P. Bernardi, V. Peronilli and D. Di Lisa. *Biomedical Sciences, University of Padova, Padova, Italy and **Biological Chemistry, University of Padova, Padova, Italy. Sponsor: **D. Fox.

We are studying the properties of the mitochondrial permeability transition pore (PTP) in cells and tissues, and we describe here our results on the relationships between PTP opening and cell death in two models. (i) We have investigated the relationship between PTP opening, mitochondrial depolarization, cytochrome c release and occurrence of cell death in MMH1C1 hepatoma cells treated with a Ca2+ ionophore or arachidonic acid, a potent PTP inducer. We observed both transient and long lasting PTP openings. While cell viability was hardly affected by PTP openings of short duration, longer PTP openings caused mitochondrial depolarization followed by release of cytochrome c and cell death. Thus, modulation of PTP open time appears to be the key element in determining the outcome of stimuli that converge on the PTP (ii) We have studied the role of the PTP in ischemia-reperfusion damage of the heart by investigating metabolism of NAD+, which is released from mitochondria when the PTP opens and then hydrated by glycolysis, and three related enzymes: LDH, lactate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase. The addition of 0.2 mM cyclosporin A (Cys A) or of its analogue Manu-4-Cys maintained higher NAD+ contents, especially in mitochondria, and protected the heart from reperfusion damage. Thus, PTP opening in the heart can be documented as a Cys A-sensitive release of NAD+ which is partially degraded by glycolysis and partly released when sarcoplasmic integrity is compromised. Once released from the matrix, NAD+ could be transformed into cyclic ADPribose, thereby promoting Ca2+ release from intracellular stores and amplifying the effects exerted by an initial rise of Ca2+.

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

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

Developmental lead exposure in rats and mice produces a progressive loss of rod photoreceptors that correlates with an apoptotic mechanism driven by retinal cell and calcium overload. We used wild-type and transgenic mice overexpressing Bcl-xL only in photoreceptors to examine the influence of Bcl-xL on mitochondria in lead-induced apoptosis. Our previous in vivo data demonstrated that Bcl-xL exerts its cytoprotective effect at the mitochondrial level. We accumulated evidence that the mitochondrial permeability transition (MPT) is the apoptotic mechanism in wild-type mice exposed to lead and is blocked by Bcl-xL overexpression. This is the first in vivo evidence that Bcl-xL overexpression regulates apoptosis. Because the MPT is involved, alterations in mitochondrial ultrastructure are expected. We used electron microscope tomography to investigate at high resolution the three-dimensional (3-D) structure of red mitochondria in retinas of wild-type and Bcl-xL mice. We analyzed more than 50 tomographic reconstructions under four conditions: wild-type control, wild-type lead-exposed, Bcl-xL transgenics and Bcl-xL lead-exposed. At a resolution of 5.7 nm, the only detectable structural modifications produced by developmental lead exposure involved the mitochondrial contact sites. No mitochondrial swelling or rupture of membranes were observed. The surface area and density of contact sites in the apoptotic mitochondria were about twice that of the control mitochondria and the Bcl-xL mitochondria both with and without lead exposure. Coupled with our biochemical and morphological assays, this structural work shows that Bcl-xL overexpression in photoreceptors provided complete protection against developmental lead-induced, rod-selective alterations to mitochondrial function and structure. Supported by NIH Grants ES03183, EY06891, EY09213 and RR04050.

18 IMAGING OF THE MITOCHONDRIAL PERMEABILITY IN MODELS OF NEUROTOXICITY.

M. A. Philbert, Environmental Health Sciences, University of Michigan, Ann Arbor, MI.

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

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

L.J. Lemasters, Cell and Developmental Biology, University of North Carolina, Chapel Hill, NC.

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

20 REGULATION OF ARYL HYDROCARBON RECEPTOR-MEDIATED SIGNAL TRANSDUCTION.

A. Dugas, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH.

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

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

G. H. Perdue and J. R. Petrusis, Department of Veterinary Science and the Center for Molecular Toxicology, Penn State University, University Park, PA.

The unliganded mouse aryl hydrocarbon receptor (mAHR) is a cytoplasmic, tetrameric complex consisting of the AhR ligand binding subunit, a dimer of heat shock protein 90, and the hepatitis B virus X associated protein 2 (XAP2). XAP2 increases mAhR localization in the cytoplasm. Studies were performed to determine the mechanism of cytoplasmic retention of the mAhR/XAP2 complex. In the presence of XAP2, the mAhR is nuclear, in the absence of XAP2, the mAhR is cytoplasmic. XAP2 interacts specifically with the dynamic ligand independent nucleocytoplasmic shuttling. One possible mechanism is through anchoring of the mAHR/XAP2 complex to the cytoskeletal matrix. This possibility was tested by disruption of both tubulin and actin networks using colchicine and cytochalasin B, respectively, with no effect on subcellular localization of the AhR complex was observed. Using additional NLSin (clear Localization Sequence) was added to the mAHR/YFP fusion protein and fluorescence microscopy revealed that AhR/YFP-NLS was no longer retained in the cytoplasm in the presence of XAP2, suggesting that XAP2 does not tether the receptor complex. A second possible mechanism of cytoplasmic retention of the mAHR by XAP2, is through blocking access to, or altering the conformation of, the bipartite NLS. The former possibility was tested by the use of an antibody directed against the mAHR's NLS and the results indicated that the NLS is accessible even in the presence of XAP2 in the core receptor complex. Whether or not XAP2 alters the conformation of the bipartite NLS leading to reduced importin binding remains a possibility. The
human AhR (hAhR) has been shown to differ biochemically from the mAhR. The ability of XAP2 to influence localization of the hAhR was examined. Results suggest that XAP2 does not influence localization or nuclear-cytoplasmic shuttling of the hAhR. Additionally, XAP2 was unable to enhance hAhR levels, in contrast to what has been observed with the mAhR.

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

C. J. Ellerink. *Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX.* Sponsor: K. Pollenz.

The aryl hydrocarbon receptor (AhR) belongs to the basic helix-loop-helix/PAS family of transcription factors that regulate critical functions during development and tissue homeostasis. Within this family, the AhR is the only member conditionally activated in response to ligands. The ligand-dependent interaction of AhR with the retinoblastoma tumor suppressor protein (pRb) interacts directly with the AhR through two distinct receptor domains spurred our interest in AhR-mediated cell cycle control. We have characterized one of the binding domains. More recent evidence revealed that the cDNAs-containing the AhR coding region thus far diverged from the AhR coding region. The cDNAs-containing the AhR coding region thus far diverged from the AhR coding region.

**23**

**IMPACT OF AH RECEPTOR DEGRADATION ON GENE TRANSCRIPTION.**

Q. Ma. *Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, NIOSH/CDCE, Morgantown, WV.*

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

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

R. S. Polletta, Z. Song and S. Dabirshahzadhebi. *Biology, University of South Florida, Tampa, FL.*

The Ah receptor (AhR) is a modular protein containing distinct amino acid motifs that confer function to the receptor. Studies have revealed that the AhR contains both nuclear localization (NLS) and nuclear export (NES) signals that control in part, the subcellular location of the AhR and may influence the ability of the AhR to be activated. Thus, studies were performed to examine the relationship between the nuclear export of the AhR and AhR-mediated gene regulation. Blockage of nuclear export in human HepG2 cells with leptomycin B (LMB) resulted in a ligand-mediated increase in the level of AhR/ARNT complex in the nucleus and correlated reductions in agonist-stimulated AhR degradation. However, despite the presence of high levels of the AhR/ARNT dimmer, induction of numerous AhR-responsive reporter genes and endogenous CYP1A1 was reduced by 78-89%. To determine whether there was a direct relationship between blockage of export of the AhR and the reduced levels of gene regulation, stable cell lines were produced that expressed an AhR with a mutant NES. Immunochemical and biochemical analysis of these cells confirmed the observation that expression of a wild-type AhR protein showed that mutation of the NES did not affect the ability of the cells to induce endogenous CYP1A1 following ligand exposure. To confirm these results and evaluate the temporal aspect of the signaling pathway, wild-type and NES mutant AhR protein were produced in vitro and then microinjected directly into the nucleus of various culture cells. While export was blocked, the injected cell line demonstrated high levels of CYP1A1 following agonist exposure. These findings demonstrate that it is possible to generate an AhR protein defective in nuclear export that functions in agonist-mediated gene induction. This implies that the negative effect of LMB on gene induction is independent of the nuclear export of the AhR and involves a novel factor that must interact with the AhR-mediated signal transduction pathway. Supported by NIEHS grants ES89880 and ES110491.

**25**

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


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

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**EMEA GUIDELINES.**


The Note for Guidance on Repeated Dose Toxicity was released in July 2000 by the CPMP (www.emea.eu.int/pdfs/human/swp/104299en.pdf). This Note for Guidance was revised to update the guidance on immunotoxicity. Immune toxicity screening is incorporated in the Note for Guidance in accordance with the tiered testing approach, i.e., an initial screening phase and extended studies. The tiered testing approach is considered sufficiently reliable to be used in a regulatory setting. Tiered testing strategies have been developed to assess direct immunotoxic effects (suppression or stimulation). Hypersensitivity or testing for autoimmune potential is not in the scope of this Note for Guidance. In attempting to prevent an increase in animal use and number of studies the SWP decided not to issue a separate guideline on immunotoxicity, but to update the Note for Guidance on Repeated Dose Toxicity. As a result, a tiered approach to toxicity testing has been evaluated and reviewed. The first tier includes immunotoxicity screening. The initial screening phase is designed to enhance the sensitivity of standard toxicity testing for immune toxic effects, preferably without the use of satellite animals. The NK-cell activity assay and lymphocyte subset phenotype analysis are selected because they fulfill these criteria. As an alternative, the primary antibody response to T-cell dependent antigen is suggested which allows a more holistic view of the immune system. For the interpretation of the initial immunotoxicity screening the CPMP document advocates an integrative analysis of the changes in the immune system and other types of toxicity and the health status of the test animal. If the initial screening phase suggests direct immunotoxicity, follow-on studies in animals may be warranted on a case-by-case basis to further study.
the altered immune response. The design of extended animal studies will depend on the nature of the immunological changes observed in the initial screening phase. It should include comprehensive in vivo or ex vivo assays of immune function. For guidance on hypersensitivity testing see (www.emea.eu.int/pdfs/human/swp214500en.pdf).

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

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

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

30 IN VIVO FORMATION OF CERAMIDE-LIKE N-ACYLATED AMINOPENTOLS FROM HYDROLYZED FUMONISIN B1.
to Toxicology & Mycotoxic Reseach, ARS, USDA, Athens, GA; Lehrstuhl fur Lebensmittelchemie, Universitats Wurzburg, Wurzburg, Germany and Department of Chemistry & Biochemistry, Emory University, Atlanta, GA.
Fumonisins are toxic and carcinogenic mycotoxins found in corn-based foods. Alkaline conditions such as those used for making masa from corn convert fumonisins to their hydrolyzed forms. The subchronic liver and kidney effects of hydrolyzed fumonisin B1 (HFB1), also referred to as aminopentol (AP1), and fumonisin B2 (F2) in rodents are similar and HFB1, like F2, inhibits the enzyme ceramide synthase. Fumonisin metabolism has not been shown. However, formation of the ceramide-like compound N-palmitoylaminopentol (PAP) from PAP-CoA and HFB1, by rat liver microsomes in vitro has recently been reported. PAP was more toxic (using cell death as the endpoint) than F2 or HFB1, to cultured H729 human colon cancer cells. To determine if PAP, or other N-acylated aminopentol (AAP) are formed in vivo, male rats (n=3) were given 52, 115 or 230 HFB1/day (ip injection) for 5 days. Controls (n=3) received the saline vehicle. Two rats/group were euthanized 2 hours after the last dose. Remaining animals were killed 3 weeks later. HFB1 was not toxic and did not cause microsomal kidney or liver lesions. It did, however, partially inhibit ceramide synthase, as shown by increased sphinganine and sphingosine concentrations in liver and kidney. Mass spectrometric analysis revealed a series of AAP, compounds in liver of the HFB1-exposed rats. The metabolites' acyl groups ranged in length from C14 (PAP) to C24, C24 compounds were most abundant, ranging from 0.5-2.4 mmole/g tissue, while up to 4 mole% of the other. Long chain compounds were detected in kidney and blood. The AAP, metabolites were not persistent; none were detected 3 weeks after the final dose. These findings demonstrate that HFB1 is a substrate for ceramide synthase in vivo. The effects of AAP, metabolites in animals chronically exposed to HFB1, warrant investigation.

31 FUMONISIN B1 (FB1) ALTERS OUABAIN-INSENSITIVE BUT NOT OUABAIN-SENSITIVE 31RB UPTAKE IN LLC-PK1 CELLS WITHOUT ALTERING MEMBRANE PERMEABILITY.
K. T. Riley, H. S. Yous and S. S. Bae, Toxicology and Mycotoxic RU, USDA-ARS, Athens, GA; College of Pharmacy, Chonbuk National University, Cheongju, South Korea and 2Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA.
FB1 is a fungal inhibitor of sphingolipid biosynthesis found in corn worldwide. Inhibition of ceramide synthase causes an increase in free sphingolipids and depletion of glycosphingolipids and sphingomyelin. Free sphingoid bases can modulate Na+/K+-ATPase activity and altered glycosphingolipid expression can modulate plasma membrane ion permeability and other membrane functions. FB also causes lipid peroxidation in rat hepatocytes and cultured cells. The purpose of this study was to determine if FB can alter membrane ion permeability, Na+/K+-ATPase function, or induce lipid peroxidation in LLC-PK1 cells. Altered membrane ion permeability and Na+/K+-ATPase function was assessed by comparing 86Rb (a transport analog for potassium) efflux and ouabain-sensitive and ouabain-insensitive 86Rb uptake in sub-confluent LLC-PK1 cells exposed to 20 μM FB (a minimally cyto-
toxic concentration) for 72 h. Lipid peroxidation was assessed using the thiobarbituric acid assay in cultures grown under similar conditions but in growth medium without phenol red and at 50 μM FB (a cytotoxic concentration). The results show that 201 FB induced C 72 h in no effect on 668B efflux from 868B loaded LLC-PK1 cells nor was there any increase in thiobarbituric acid substances: at 50 μM. These findings are consistent with the conclusion that under these conditions FB had no effect on membrane permeability. There was also no significant effect on ouabain-sensitive 868B uptake indicating that FB had no effect on Na+/K+ ATPase function. There was however a significant increase in ouabain-insensitive 868B uptake indicating that a minimally cytotoxic but ceramide synthesis inhibitor concentration of FB can modify potassium transport processes. A possible target is the ouabain-insensitive phospholipid-dependent 868B uptake associated with transporters such as that of the non-gastric H+/K+ ATPase.

32 FUMONISIN B1 INDUCES DECREASED EXPRESSION OF PK2 MAP KINASE IN LLC-PK1 CELLS.

Pharmaceutical & Biomedical Sciences, University of Georgia, Athens, GA; and Toxicology & Mycotoxicology, Athens, GA.

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

33 EFFECT OF FUMONISIN B1 ON PROTEIN KINASE C-α IN CULTURED RENAL LLC-PK1 CELLS.

N. V. Gopee and B. P. Sharma. Physiology and Pharmacology, The University of Georgia, Athens, GA.

Fumonisin B1 (FB1) is a ubiquitous, potent, and naturally occurring mycotoxin produced by the fungus Fusarium verticillioides. It has been implicated in fatal and debilitating diseases in both animals and humans. FB1 is known to induce cytotoxicity by disruption of de novo sphingolipid biosynthesis via inhibition of ceramide synthase and induction of numerous proinflammatory cytokines, including tumor necrosis factor α. Protein kinase C (PKC) is a serine/threonine kinase involved in a number of signal transduction pathways, including apoptosis and carcinogenesis. As sphingolipids are the natural inhibitors of PKC, it is conceivable that FB1 may affect PKC-regulated functions. FB1 has been shown to cause both activation and repression of PKC depending on the dose, cell type and PKC isoform. This study investigated the acute temporal and dose effects of FB1 on PKC isoforms in pig renal epithelial cells (LLC-PK1). Exposure of LLC-PK1 cells to 1 μM FB1 caused a rapid and transient translocation (5 min) of PKCγ from the cytosol to the plasma membrane. A time-dependent increase in the stabilization of PKCγ (15-30 min) was followed by a decrease at 120 min. PKCγ isoforms δ and ε were unaffected by FB1, at all time periods. PKCα membrane translocation increased in a dose-dependent manner with a significant increase at 0.3 μM and 1 μM FB1. Sphingolipid biosynthesis of PKCγ at FB1 concentrations 2-3 μM was observed. Pretreatment of LLC-PK1 cells with the PKC activator, phorbol 12-myristate 13-acetate (PMA) resulted in a further increase in PKCγ translocation as compared to PMA alone. The FB1-induced PKCγ translocation was reversed by preincubation of LLC-PK1 cells with 100 μM Cytosol α, an inhibitor of PKC. The serum palmitoyltransferase inhibitor, mycotoxin, did not prevent the FB1-induced translocation of PKCγ. The ability of FB1 to induce PKCγ translocation in LLC-PK1 cells and ultimately in its signal transduction pathways may have implications in its ability to produce both apoptosis and carcinogenesis. (Supported in part by NIH grant ES09403.)

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

College of Veterinary Medicine, University of Illinois, Urbana, IL and USDA/ARS, Washington, DC.

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

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

College of Veterinary Medicine, University of Illinois, Urbana, IL and USDA/ARS, Washington, DC. Sponsor: W. Haschke, Rockwell.

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

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

J. K. Kerze and A. Papa. Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH.

Cardiovascular disease, primarily associated with disorders of atherosclerotic etiology, is the main cause of morbidity and mortality in the United States. Overt and cumulative exposure to environmental or occupational toxicants may play a role in the onset and progression of this disease. More than 35 different predisposing risk factors.
factors for atherosclerosis have been identified, but little is known of the mechanism of action of environmental agents, due in part to lack of epidemiological studies and in part to lack of adequate experimental models. Dioxins, such as 2, 3, 7, 8-tetrachlorodibenzo-\(p\)-dioxin (TCDD), have been implicated as risk factors for atherosclerosis in humans. The objective of these studies was to test the hypothesis that TCDD exposure results in an aryl hydrocarbon receptor (AhR)-dependent reprogramming of gene expression in vascular smooth muscle cells (vSMCs). To test the hypothesis, we used primary cultures of vSMCs and apoptosis, as well as cyskine, chemokine and their receptors. Numerous cell cycle and apoptotic genes were found to be deregulated by 1nM TCDD in quiescent and synchronized vSMCs from Ahr\(^{-}\) mice, while not in vSMCs from Ahr mouse. TCDD increased the mRNA levels of p21\(^{\text{ras}}\), mdm2, caspase 1, and cyclin D1 compared to controls by 2.7, 2.4, 2.2, and 1.8 fold, respectively. Decreases in TCDD-mediated gene expression were observed for p15\(^{\text{ink4a}}\) and p57\(^{\text{kip2}}\) compared to controls by 2.7 and 2.3 fold, respectively. At the protein level alterations in expression were detected for mdm2, caspase 1, and p15\(^{\text{ink4a}}\). These data suggest that vascular toxicants, such as TCDD, may alter gene profiles in vSMC in AhR-dependent manner that can translate into functional changes at the protein level. This finding helps us define a molecular fingerprint of vascular toxicants of utility to reduce the use of animals in toxicity testing. (Supported by Colgate-Palmolive/Society of Toxicology Postdoctoral Fellowship and NIH 5F32ES11250-01).

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

A. K. Lund\(^{1}\), N. L. Kanagy\(^{1}\) and M. K. Walker\(^{1}\).

1. College of Pharmacy, University of New Mexico, Albuquerque, NM and 2. Cell Biology and Physiology, University of New Mexico, Albuquerque, NM.

AhR is a ligand-activated transcription factor known to mediate the toxicity of compounds such as 2, 3, 7, 8-tetrachlorodibenzo-\(p\)-dioxin (TCDD), polycyclic aromatic hydrocarbons. Upon activation, AhR dimers with the aryl hydrocarbon nuclear translocator protein (ARNT). This complex then translocates into the nucleus where it binds to response elements (DREs) and upregulates a series of xenobiotic metabolizing enzymes. ARNT also heterodimerizes with hypoxia inducible factor 1 alpha (HIF1\(\alpha\)), which then binds to hypoxia responsive elements (HREs), increasing transcription of hypoxia-induced genes. We have shown that AhR null mice develop cardiac hypertrophy by 5 months of age and exhibit increased HIF1\(\alpha\) signaling in the heart. In addition, endothelin-1 (ET-1) is upregulated by hypoxia and the HIF1\(\alpha\) signaling pathway. ET-1 is a circulating peptide associated with hypertension and cardiac hypertrophy. Thus, we tested the hypothesis that AhR null mice exhibit hypertension associated with increased plasma ET-1. Cardiac chambers were surgically placed in the femoral artery of both wildtype C57Bl6 and AhR null mice, age 4-6 months. Mean arterial blood pressure (MAP) was measured over 10 minute recording period on day 3 post surgery and then the mouse was sacrificed and blood collected. Plasma ET-1 was measured using an RIA. AhR null mice exhibited significantly higher MAP compared to wildtype (wildtype 98 ± 1.5 mm Hg, 12C 13 ± 3.0 mm Hg, p < 0.001). AhR null mice also exhibited significantly higher plasma ET-1 compared to wildtype (wildtype, 8.6 ± 1.1; null, 11.3 ± 0.7 fM/mL; p < 0.04). Thus, AhR null mice exhibit hypertension associated with increased plasma ET-1 levels. These data suggest that the increased hypertension and increased plasma ET-1 could contribute to cardiac hypertrophy in AhR null mice. Supported in part by NIH grants ES10435 and American Heart Association Desert Mountain Affiliate 0151511Z.

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

H. M. Handley\(^{1}\), M. W. Grow\(^{1}\), L. J. Scoggan\(^{1}\) and M. C. Fishman\(^{1}\).

1. Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA, 2. Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN and 3. Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA.

2, 3, 7, 8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) is a powerful teratogen that perturbs the developing cardiovascular system of zebrafish. In embryos exposed to TCDD, the heart distal to the aortic arches and the branchial arches, and therefore not yet to be defined. To address this problem, we developed zebrafish cDNA microarrays consisting of nearly 5, 200 adult heart cDNA clones, known heart markers, nuclear receptors and cytochrome P450s. Using these arrays, we have characterized alterations in embryonic gene expression resulting from TCDD exposures ranging from ED10 to ED100 for cardiovascular symptoms. Induction of CYP1A was consistently the strongest, most sensitive response. Expression of CYP1A (aromatase), AhR, and 2, 3, 7, 8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) encodes a receptor, more than thirty novel transcriptional responses were identified from among the adult heart cDNAs. By correlating the magnitude of transcriptional changes with that of toxicity, we have generated a list of genes that are likely to be involved in cardiovascular embryotoxicity. NIH #P42ES07381

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

D.E. Heck\(^{1}\), P. Zhou\(^{1}\), J. D. Laskin\(^{1}\) and C. Gardner\(^{1}\).

1. Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and 2. Envt. and Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

The ubiquitous environmental contaminant, 2, 3, 7, 8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) is a developmental toxicant, potent tumor promoter in mice, and putative human carcinogen. TCDD elicits effects through altering gene expression in susceptible cells. We have found that exposure to TCDD results in activation of the interferon-gamma (IFN-\(\gamma\)) activated sequence (GAS). Binding of IFN-\(\gamma\) to its cellular receptor initiates tyrosine phosphorylation of STAT proteins, dimerization and subsequent translocation to the nucleus through interactions with a combination of second-messenger molecules, resulting in activation of GAS elements in the promoter regions of IFN-\(\gamma\)-sensitive genes. Activation of GAS elements plays an important role in modulating expression of an array of genes important in inflammation. We have found that, similar to IFN-\(\gamma\), TCDD induces expression of type 2 nitric oxide synthase, type 1 MHC, and COX-2 in rat hepatic endothelial cells. The toxicity of TCDD is mediated largely through binding of the environmental toxicant to the Ah receptor. We have found that the TCDD-bound Ah receptor directly interacts with STAT1 modifying its activity. Moreover, in a manner similar to that of IFN-\(\gamma\), TCDD-mediated induction of gene expression results in functional alterations to hepatic endothelial cells. Nitric oxide production and proagangulon reassembly were modified in endothelial cells following TCDD- treatment. Through regulation of STAT1, NOS2, type II major histocompatibility antigen, cyclooxy- genase 2 and other proinflammatory proteins, endothelial cells become immunologically activated, a process that plays an important role in inflammation and tissue injury. We do not suggest that TCDD binds to AhR nuclear translocator (ARNT), binds dioxin response elements, and upregulates xenobiotic metabolism genes. In response to hypoxia, ARNT also heterodimerizes with hypoxia-inducible factor 1 alpha (HIF1\(\alpha\)). The HIF1\(\alpha\)/ARNT complex then binds to hypoxia responsive elements, and facilitates transcription of hypoxia-responsive genes such as vascular endothelial growth factor (VEGF). This suggests that cross-talk between the TCDD and hypoxic signaling pathways could exist within the heart. We have previously reported that AhR null mice develop cardiac hypertrophy by 5 mo, as measured by increases in heart weight, heart/body weight ratio, and the cardiac hypertrophy markers, beta-myosin heavy chain and myosin light chain-2V. Here we show that AhR-/- mice have increased HIF1\(\alpha\) protein and upregulate the hypoxia-responsive gene VEGF at 5 mo. We then used M isozyme (MISO), which binds regions in a hyperglycemic-dependent manner, to determine the relative oxygen concentrations in wildtype and AhR-/- mice. At 3 mo, a time when no significant increase in heart weight nor HIF1\(\alpha\) protein is seen in AhR-/- mice, no increase in MISO binding was observed. At 5 mo, when AhR-/- mice show a significant increase in heart weight and HIF1\(\alpha\) protein, there was still no increase in cardiac MISO binding. These data show that AhR-/- mice develop a significant increase in signaling through the cardiac HIF1\(\alpha\)-mediated pathway despite nonoxic conditions within the heart. This suggests that the increase in cardiac HIF1\(\alpha\) signaling may be caused by deregulation of the HIF1\(\alpha\)-mediated pathway due to a loss of AhR within the heart, and not by impaired oxygenation of the myocardium. Supported by NIH ES08881 and AHA Heart Association Affiliate 0151511Z.
Environmental hydrocarbons have been implicated as significant risk factors in human atherosclerotic vascular disease. Using benz[a]pyrene (BaP) as a model compound, this laboratory has shown that interference with gene transcription is a critical event in the induction of proliferative (i.e. atherogenic) phenotypes in vascular smooth muscle cells (vSMCs). The complexity of the cellular response elicited by BaP requires characterization of interactive gene networks involved in the atherogenic response. To achieve this objective, vSMCs isolated from the thoracic aorta of adult C57BL/6J were established in serial culture and used as a model to evaluate the atherogenic response to BaP. G0 synchronized cultures were released into growth by addition of fetal bovine serum in the presence of DMSO (control) or 3 μM BaP for 8 hr, a regimen known to stimulate vSMC proliferation (1). Controls and treated mRNAs were isolated and labeled with Cy3 and Cy5, respectively, and hybridized to custom-made mouse cDNA microarrays of 960 genes spotted in triplicate. Array intensities were evaluated as prescribed by GeneSpring software to identify differentially expressed genes. Over 150 genes were altered in vSMCs by BaP treatment. Genes coding for RAS, cholesterol-regulating, ERK6-protein, several heat shock proteins, retinoic acid-repressible protein, and insulin-like growth factor 1 receptor were significantly upregulated. Other genes increased by atherogenic treatment included peroxiredoxin, coflin, bronchodilator PHD finger transcription factor, TIF1 beta, GTP-binding protein, G protein gamma-2 subunit, reca/RAD51, and BAF53a. These results confirm previous findings and implicate genes involved in ras signaling, oxidative stress, DNA repair, and chromatin remodeling in the atherogenic response elicited by BaP (Supported by NIH grants ES 04849 and ES09106). CD1 is a postdoctoral fellow in NIEHS training grant ES 07273).

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

J. M. Martines, C. A. Afshari, C. R. Miller and N. L. Walker, NIEHS, Research Triangle Park, NC.

Interferon (IFN) can play a role in development of primary tumor and shape tumorigenicity. Previous results using real-time RT-PCR and microarray analysis of human peripheral lung airway epithelial cells (HPLA1A), showed repression of several IFN regulated genes and induction of IFN regulatory factor 4 (IRF4) when treated with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). We hypothesize that TCDD alters the response of HPLA1A cells to interferon. Since IFN-gamma is known to inhibit cell proliferation, cells were treated with 1X104 units/ml for 24 hours plus or minus TCDD (10μM) pretreatment. An aqueous soluble tetrazolium/formazan assay was used to detect cell growth changes. Up to 30 nM concentrations, TCDD did not cause any cell growth changes compared to cells given vehicle (PBS) only. By comparison, treatment with IFN-gamma caused a 28% decrease in cell growth (P < 0.001). Pre-treatment with TCDD (10μM), reduced this decrease in cell growth caused by IFN-gamma by approximately 50% (P < 0.001).

Our next objective was to do a species comparison looking at in vivo gene expression in rat lung. We sought to determine how TCDD treatment affected interferon regulatory factor 4 (IRF4) and mumps virus (influenza) resistance 1, homolog of murine interferon-inducible protein p78 (MX1). Sprague-Dawley rats were treated by oral gavage twice weekly. Control animals were given vehicle (corn oil) only, and the treated group consisted of a substrate treatment (350 mg/kg twice a week). Using RNA isolated from rat lung tissue, we analyzed IRF4 and MX1 expression by real-time RT-PCR. We found that IRF4 was induced after 3 weeks TCDD treatment, while MX1 was suppressed. This inverse gene relationship was the trend of expression we previously found in the HPLA1A cell line. Overall, this data suggest a novel role for TCDD in immunomodulation in human lung cells that appears to be conserved in rats.

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

E. S. Williams, A. Kidafl$, E. Wilson and K. S. Ramos, Center for Environmental and Rural Health, Texas A&M University, College Station, TX and 1Health Science Center, University of Texas, Houston, TX.

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

44 TOXICOCHEMOMICS: FINGERPRINTING TOXIC COMPOUNDS USING GENE EXPRESSION PROFILES.

L. Suter-Dick, F. Boess, R. Gasser, C. de Vera, P. Crameri, M. Bedoucha, S. Evers and S. Albertini. PRINS/Toxicology, F. Hoffmann-La Roche, Basel, Switzerland.

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

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

T. Hansen$, M. Dangers$, J. Borlak$, B. Germsmayer$, S. Tomiuk$, K. Hofmann$, F. Hube$, and A. Bosio$. 1MEMORIE Sonnolog GmbH, Cologne, Germany and 1Centre for Drug Research and Medical Biotechnology, Fraunhofer Institute ITA, Hanover, Germany, Sponsor: H. Malle.

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

46 GENE EXPRESSION CHANGES IN RAT LIVER ASSOCIATED WITH CHOLESTASIS.

Microarray technology enables the investigation of large scale gene expression in response to toxic insult. By generating expression data on many genes simultaneously it is feasible that compounds could be classified according to their gene expression pattern or "signature." The aim of the following work was to determine whether different mechanisms of hepatotoxicity could be distinguished based on differential gene expression analysis. Oestrogens and oestrogen glucuronides have been chosen specifically for the purpose of this study as they have long been implicated in the pathogenesis of intrahepatic cholestasis. To complement these, one structurally-related oestrogen that does not cause cholestasis and the model hepatotoxin alpha-naphthyl isothiocyanate and carbon tetrachloride have been added for comparative purposes. Male Sprague-Dawley rats, 250-355 g were administered with either 17-alpha-ethinylestradiol, estradiol-17-beta-D-glucuronide, oestradiol-17-beta-D-glucuronide, oestradiol-16-alpha-(beta-D-glucuronide) or oestradiol-3-beta-D-glucuronide at 5mg/kg/day. Alpha-naphthyl isothiocyanate and carbon tetrachloride were administered at 50mg/kg/day and 100mg/kg/day respectively. Compounds were administered to five animals per compound group for a series of four days followed by necropsy on day five. The liver from each animal was immediately snap frozen in liquid nitrogen and stored at -80°C. Following this, total RNA was extracted and gene expression analysis carried out using a proprietary cDNA microarray system. Six replicate microarray hybridizations were performed for each RNA sample and the data were analysed by univariate and multivariate analysis. The results showed a difference in gene expression associated with different treatments groups. The prototype of this technology is useful for gene expression pattern profiling associated with different hepatotoxins and may facilitate predictive toxicology.

47 DNA MICROARRAY ANALYSIS OF PROTOTYPE HEPATOCELLULAR CARCINOGENS IN PRIMARY RAT HEPATOCYTES.
K. L. Kolaiz, J. A. Kramer, R. T. Bunch, S. W. Curry, C. Jackson, D. L. Morris and J. C. Davila. 'Global Toxicology, Pharmacia, Stokke, IL, and 'Global Investigative Toxicology, Pharmacia, St Louis, MO.

An in vitro study was performed using primary rat hepatocytes to evaluate the gene expression profiling of known rodent hepatocarcinogens. Adult rat hepatocytes were cultured with Matrigel and treated with 2AAF, 4AAF, benzo[a]pyrene (B(a)P), dialkylamine (DOX), methylcholanthrene (MCT), tamoxifen (Tam), and phenobarbital (PHN) for 48 hr at concentrations ranging from 1 to 900 nM. Doses determined to be toxic by MTT reduction and LDH leakage were not evaluated for gene expression. Messenger RNA was isolated from cultured hepatocytes and used to prepare Cy3- and Cy5-labeled cDNA probes. The probes were hybridized against Incyte RarGem 1 microarrays, which contain approximately 7,800 arrayed sequences. Several approaches were used to analyze the profiling data, statistical evalution, including gene clustering, correlations with phenotypes, and pathway analysis. Characteristic P450 induction profiles were observed as 2AAF, 4AAF, B(a)P, MCT, and TAM treatment induced CYP1A1; 4AAF, B(a)P, and PB treatment induced CYP2B1; and 2AAF, 4AAF, B(a)P, DOX, TAM and PB induced CYP3A1. Clustering the expression data was utilized to identify common molecular markers that may be predictive of carcinogenicity and of genotoxicity such as thymosin beta 4. Interestingly, clustering analysis results from in vitro treatment of these same compounds led to a completely different subset of genes identified after in vivo treatment.

48 INVESTIGATION OF THE GENE EXPRESSION PROFILES OF HEPATOTOXIC AND NON-HEPATOTOXIC THIOXANTHONE ANTI-TUMOR AGENTS USING cDNA MICROARRAYS.
L. T. Roadcap, V. D. Shultz, T. E. White and A. E. Roberts. Toxicology, Sanofi- Synthelabo Research, Marlboro, MA.

Gene array technology may prove useful for investigating molecular mechanisms and screening new drug candidates. The utility of microarrays will depend on the extent to which they can identify and predict the genes that are changed in target organs. Sanofi-Synthelabo is studying the utility of microarrays using a series of 5 thioxanthenone anti-tumor agents. Within this series of DNA-damaging anti-tumor agents there are both hepatotoxic (3) and non-hepatotoxic (2) compounds. The gene expression profiles of these compounds are being compared as a means of identifying genes associated with hepatotoxicity. HepG2 human hepatoblastoma cells were exposed to each thioxanthenone at a concentration that results in 20-30% cell death (MTT reduction). Cells were exposed to compound for a period of 4 hours to approximate the in vivo half-life of the thioxanthenes. Cells were incubated for 44 additional hours to allow time for appearance of hepatotoxicity in vivo. mRNA was isolated and labeled cDNA probes were generated and hybridized to GeneChip Human Toxicology HT™ arrays. The expression of 144 genes was altered by the thioxanthenes (≥1.5-fold increase or decrease over untreated controls). Genes whose expression was changed included those involved in apoptosis, cell signaling, cell cycle regulation, DNA damage and repair, stress response, and xenobiotic metabolism. Of the total list of changed genes, 10 were changed only by hepatotoxic thioxanthenes (e.g., GST homolog). 17 others were changed only by non-hepatotoxic thioxanthenes (e.g., ERK4, MAPK4). As expected for DNA-damaging agents, genes such as p53, WAF1/p21, GADD153, GSFH1, c-jun, and ERCC1 were changed by all 5 thioxanthenes. These results suggest there are gene expression changes specific to hepatotoxic thioxanthenes. A more extensive time course analysis of gene expression is being performed to determine the molecular pathways associated with the onset of hepatotoxicity.

49 GENE EXPRESSION PROFILES OF 4-AMINOBIPHENYL AND BIPHENYL IN MOUSE LIVER.
C. A. McQueen, B. Chau, V. E. Richards and G. Watts. 'Pharmacology and Toxicology, University of Arizona, Tucson, AZ and "Arizona Cancer Center, Tucson, AZ.

 Arylamines such as 4-aminobiphenyl (4ABP) are human and animal carcinogens. Bio-transformation results in the formation of electrophilic products that can react with DNA causing mutations leading to the development of tumors. While the genotoxicity of 4ABP is well defined, little is known about alterations that may occur in the expression of genes in target tissue. A study was initiated to profile the changes in gene expression associated with acute exposure to the carcinogen 4ABP. The noncarcinogenic structural analog biphenyl (BP) was also tested in order to assess the contribution of reactive arylamine products to changes in expression patterns. Adult male C57Bl/6 mice were given an oral dose 120 mg 4ABP or BP/kg. Controls received corn oil. After 24 hr, livers were harvested. RNA was isolated and used to synthesize cDNAs labeled with Cy3 or Cy5. Labeled cDNAs from control and treated animals were competitively hybridized to a 5300 microarray created using mouse cDNA clones obtained from Research Genetics, then scanned to detect fluorescence intensities. Data were examined based on 3 replicate experiments. Initial analysis at p<0.01 identified 270 genes with 4ABP-induced changes in expression and 59 with BP. With the additional stringency of a 2 fold change in expression, 60 genes were altered with 4ABP while BP induced no effect. An alternative analysis based on a 2 fold change in expression ratio and a minimum fluorescence intensity of 200 also showed no changes with BP exposure but 4ABP-induced changes in 124 genes. There were 55 genes up regulated and 69 down regulated. These included genes related to stress responses such as MT-1, TGF-beta and Grp78. 4ABP and BP differed in the number of genes in the two groups that reached the 2 fold position, generated compound specific gene expression profiles. These data support the conclusion that the increased number of genes affected by 4ABP is linked to its potential for further activation and formation of electrophilic products. (Supported by ES 05912 and ES 10047).

50 ASSESSMENT OF HEPATOCYTES AND LIVER SLICES AS IN VITROTE SYSTEMS TO PREDICT IN VIVO GENE EXPRESSION.
J. Stahil, A. de Peyster, B. Jessen and G. Stevens. 'Graduate School of Public Health, San Diego State University, San Diego, CA and Drug Safety Evaluation, Pfizer Global R&D/Detectour Pharmaceuticals, San Diego, CA.

Primary hepatocytes and precision cut liver slices are two common in vitro systems used to predict and evaluate xenobiotic induced hepatotoxicity. Toxicogenomics has recently been used to assess changes in gene expression in a variety of in vivo and in vitro systems. However, comparisons of changes in gene expression in response to toxicant exposure in vitro to those observed in vivo have not been performed. This study was conducted in rats to assess the changes in gene expression in primary hepatocytes, precision cut liver slices and in vivo after exposure to known hepatotoxins. Five representative compounds, phenobarbital (PB), carbon tetrachloride (CCl4), Wy-14, 634, alpha-naphthylisothiocyanate (ANIT) and tacrine were selected for their broad range of mechanisms in hepatocellular response. In vivo, male rats (4/group) were treated with acute doses of PB (100 mg/kg), CCl4 (1ml/kg), Wy-14, 634 (150 mg/kg), ANIT (150 mg/kg) or tacrine (30 mg/kg) and sacrificed 24 hours later. In vitro, hepatocytes and liver slices were incubated with
PB (0.1 mM), CCl₄ (0.5 mM), Ws-14, 634 (0.5 mM), ANIT (0.1 mM) or tetrane (0.05 mM) for 24 hours. Total RNA was extracted from the three test systems and gene expression measured using Affymetrix U34A rat GeneChips. Very few genes that change with in vitro treatment were observed in either of the two in vitro systems. In contrast, the genes expressed in tissue slices did appear to provide a better correlation to the in vitro setting than hepatocytes. Additional time points and doses (or concentrations) will be required to elucidate the true correlation between the models.

51 GENE EXPRESSION PROFILING REVEALS MULTIPLE TOXICITY ENDPOINTS INDUCED BY HEPATOTOXINS.
Toxicology, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT.

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

52 ASSESSMENT OF HEPATOTOXIN-INDUCED DIFFERENTIAL GENE EXPRESSION IN VITRO USING cDNA MICROARRAY TECHNOLOGY.
H. M. Harries, C. M. Duggan and V. A. Baker. SEAC, Univeks, Bedfordshire, United Kingdom. Sponsor: D. Baskett.

Toxigenomics is a powerful tool for investigating the mechanisms of toxicity and predicting adverse effects by monitoring large-scale gene expression profiles. However, it is necessary to validate the data generated using microarray technology with compounds of well characterized toxicity. Hepatotoxicity, being one of the most well researched toxicity endpoints, was chosen for this purpose. HepG2 cells were exposed (in triplicate) to four classic hepatotoxic carbon tetrachloride (CCL₄), clofibrate, ethanol (EtOH) and dimethylformamide (DMF) for periods of 2h and 24h at high and low doses corresponding to 50% and 20% cell death (as determined by MTT assay) respectively. Following exposure, mRNA was isolated. cDNA labelled cDNA probes constructed and used to probe Toxicology II arrays (Cho et al., 2002). Probe arrays were exposed to X-ray film, and the images captured and analysed using AtlasImage software. Data revealed relatively few changes in gene expression following 2h exposure compared to 24h exposure, this being more pronounced at the higher doses. Although there was some similarity in differential gene expression observed across chemicals, a number of hepatotoxic-specific gene changes were identified, reflecting different mechanisms of toxicity. For example, treatment with EtOH (both concentrations) gave rise predominantly to differential expression of genes involved in metabolism (e.g. CYP3A3) and stress response (e.g. hsp cristallin A4), whereas CCL₄ (both concentrations) resulted in gene expression changes involved in extracellular (e.g. OCLN) and cell signalling (e.g. tyrosine kinase receptor). Real Time PCR was used to confirm expression for selected genes. In conclusion, these results suggest that specific patterns of gene expression may be associated with different mechanisms of hepatotoxicity, which may be useful as early markers of toxicity.

53 GENE EXPRESSION PROFILING OF CULTURES OF PRIMARY RAT HEPATOCYTES TREATED WITH HYDRAZINE.
Chemistry, United States Air Force Academy, Colorado Springs, CO, Biochemistry and Molecular Biology, Wright State University, Dayton, OH, MaxTech Environmental Technology Inc., Dayton, OH and Air Force Research Laboratory, Wright-Patterson AFB, OH.

Hydrazine and its derivatives are colorless liquids used by the Department of Defense as reducing agents in aircraft and propellant systems. Various toxic effects have been associated with exposure to hydrazine, including skin irritation, convul-

sions, hepatotoxicity, hemolytic effects, neurotoxicity, genotoxicity and it is also a suspected carcinogen. However, there are limited data on genotoxic effects related to hydrazine exposure. In this study, primary cultures of rat hepatocytes were treated with 25, 50 or 50 mEq hydrazine for 4 hours or 24 hours. Total RNA was isolated immediately following the exposure to determine mRNA expression profiles using the Affymetrix RatTox U34 and the Affymetrix RatGenome U34A arrays. Our results indicated that hydrazine exposure caused a significant induction of stress related genes (e.g., hsp70 and hsp60), transcription factors such as AP-1 and Sp-1 and a modest decrease in expression of various cellular proliferation growth factors (e.g., VEGF, IGF-1, EGF and IGF-3), but a significant (more than 2-fold) down-regulation of certain phase I and phase II drug metabolizing enzymes (e.g., CYP 450s, GSTs and NQO1). In addition, a significant down-regulation of the stress related homa oxygenase gene was seen. These results will provide important data for a complete risk assessment for hydrazine and serve as a baseline for evaluating the toxicity of newly synthesized high-energy chemicals as candidate replacements for hydrazine.

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

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

55 TRANSCRIPTOMIC AND PROTEOMIC FINGERPRINTING OF HYDRAZINE TOXICITY IN PRIMARY RAT HEPATOCYTES.
MaxTech Environmental Technology Inc., Dayton, OH, "TIPU, Columbus, IN, "Air Force Institute of Technology, WPAB, OH and "AF/HALS/HET, WPAB, OH.

Any toxic chemical's will disturb gene expression and will give rise to a specific "fingerprints" of chemical toxicity. These changes in gene expression are extremely sensitive and unique, and can serve as biomarkers of the ultimate toxicity. Hydrazine (H2) is used as fuel and propellant in aircraft and rockets. H2 is a hepatotoxic chemical, causing cirrhosis and necrosis in liver and is a suspected carcinogen. There are limited data on the mechanisms of H2 toxicity, particularly on how H2 affects gene expression. To fingerprint H2 toxicity, rat primary hepatocytes were exposed to H2 at 0, 50 or 75 mM for 2 hrs at 37°C. Total RNA was isolated at -2 (pretreatment), 0, 3, 6, 12 and 24 hr post exposure to determine mRNA expression profiles using the Affymetrix RatTox U34 array. Cell lysates were also prepared at the same time points to determine global protein expression by 2d gel electrophoresis followed by peptide fingerprint and mass spectrometry. Our results indicated that (1) H2 modulated gene expression profiles and different fingerprints were observed for certain genes; (2) effects of H2 on gene expression profiles in rat primary hepatocytes were consistent with the results of cytotoxicity data, however, transcript responses seemed to be more sensitive; (3) based on approximately 110 proteins identified out of 1400 spots on 2d gels, protein expression was not well
correlated to the corresponding mRNA expression. (4) In situ modulation of transcripts resulted in time-specific patterns for certain genes, with an immediate significant induction of stress genes (i.e., hsp70b and hsp70a), but a delayed dose-dependent up-regulation of apoptosis genes (i.e., caspase-2 and fos) suggesting possible delayed apoptosis of cells. The transcriptomic and proteomic fingerprints of hsp toxicity in primary rat hepatocytes can help in identifying early biomarkers that can be used to predict human health effects.

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


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

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

S. Kuttner, E. J. Neafsey and M. A. Collins. Cell Biology, Neurobiology and Anatomy, Loyola University Medical Center, Maywood, IL.

Binge alcoholism results in brain damage. Though brain damage was suggested as a result of synaptic excitotoxic events, the underlying mechanisms are uncertain. Earlier results using the diuretic Furosemide (Faseb J. 12:221; 1998) suggest that non-synaptic regulation of brain cell volume may be more important for the occurrence of neuronal degeneration due to binge alcohol abuse than activation of synaptic excitatory receptors. As Furosemide is an efficient anti-oxidant agent, the protective effect of Furosemide was related to its anti-oxidant activity rather than reduction in edema (Flament et al., Soc. Neurosci. Abst. 2000). To test the essentiality of brain edema in binge ethanol induced brain damage, the diuretic drug acetazolamide (AZT) was used. AZT, a carbonic anhydrase inhibitor, is a different class of diuretic compared to Furosemide (Na/K/Cl channel inhibition), AZT is not an anti-oxidant as evidenced by the oxygen radical absorbance capacity (ORAC) assay. Male Wistar albino rats were separated into four treatment groups: control (saline), AZT (30 mg/kg b.w. i.p.), ethanol (EHT; 30% orally gavaged), ETOH+AZT. Rats were treated for 8 days. Blood ethanol levels were comparable and insignificant in ethanol treated rats and ETOH+AZT treatment group. Brain edema was quantified following the method outlined by Elliot and Jasper (1949). AZT significantly (p<0.01) reduced brain edema in ETOH+AZT treated rats compared to ETOH treated rats. In addition to blocking brain hydration, AZT treatment reduced degeneration in ETOH intoxicated rats. The protective effect of the diuretic AZT was also confirmed in an in vitro model of binge ETOH toxicity using organotypic hippocampal-entorhinal cortex culture. The results of the in vitro study in tandem with in vivo organotypic culture suggest brain edema as an essential upstream event leading to brain damage independent of the anti-oxidant activity of the diuretic. Further studies aimed at quantitative analyses of brain edema in organotypic cultures are under way.

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

J. L. Tobias and R. T. Miller. Graduate Center for Toxicology, University of Kentucky, Lexington, KY.

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

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

A. Shimada, M. Maeda, Y. Mizutani, M. Sawada and T. Morita. Veterinary Pathology, Tohoku University, Tottori, Japan.

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

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

K. A. Hogan and P. K. Sonwali. 1 Toxicology, Rutgers Univ., New Brunswick, NJ and 2 Neurology, UMDNJ-RWJMS, Piscataway, NJ.

Methamphetamine (METH) is a synaptic toxin in experimental animals. Neurochemical indices demonstrate loss of nerve terminal integrity in animals and humans following toxic doses of METH. Studies in human abusers with acute
METH intoxication report no decrement in postsynaptic measures of [3H]-dihydropyridine receptor (DTBZ) binding to the neuronal vesicular monoamine transporter (VMAT2). This finding in humans, which is unexplored in mice, is unexpected in light of significant decrements observed in other neurochemical indices. In the present study, [3H]-DTBZ binding was examined in cortical homogenates of MET-treated mice at time points between 1-85 days post-MET. These studies reveal that VMAT2 binding in stratal homogenate may not be a reliable index of nerve terminal loss at early time points (1-4 days) following METH intoxication. In contrast to significant loss of other neurochemical indices [3H]-DTBZ homogenate binding gradually declined. On day 1 [3H]-DTBZ homogenate binding was compared to [3H]-DA uptake in -striatal homogenate, which is consistent with the early compromised of DAT (77%) and TH (65%). These results suggest that [3H]-DTBZ binding in homogenate may not adequately reflect nerve terminal status at early time points. However, at time points after day 4, less of [3H]-DTBZ binding in homogenate (60%) closely paralleled loss of TH activity (60%). Over the course of 85 days, loss of [3H]-DTBZ binding and TH activity were not as substantial as that of DA binding and DA content. Recovery of DA content occurred at the same rate; although measures demonstrating recovery persisted below control values, indicating a lasting neurotoxic effect in METH-treated mice.

61 THE TRIGGERING ROLE OF DOPAMINE IN NEUROTOXIC DAMAGE CAUSED BY AMPHETAMINE.
The breakdown products of dopamine (DA) are potentially neurotoxic but neurotional damage due to amphetamine (Amph) treatment may not only be produced by DA radical scavengers. This study was aimed to explore the relationships between extracellular Da, glutamate (Glu) and aspartate (Asp) and hydroxyl radical (OH) generation during subchronic Amph treatment (5 mg/kg, 4 injections i.p. with 2-hour interval). The extracellular levels of DA, Glu and Asp were estimated using means of HPLC and the generation of OH during slices were estimated in the extracellular fluid using FAD as an indicator. Amph caused an immediate increase in the extracellular DA concentration up to 156%, which effect was quickly reduced to the baseline values. The subsequent Amph injections were followed by a much smaller increase in the extracellular DA concentration (about 300%). Amph produced marked increase in the OH generation, the first wave was observed 80 min after the second injection and persisted during 2 hours (up to 700%), and the second increase occurred after the fourth injection (up to 400%). Amph (5 mg/kg) caused a marked gradual increase in the Glu and Tau levels (up to 500 and 450% of the pretreatment levels, respectively) by the end of experiment. Our results suggest a triggering role DA in neurochemical changes which lead to neuron damage. The changes in extracellular DA, Glu, Tau and OH reflect different subsequent phases of Amph neurotoxicity.

62 IDENTIFICATION OF A PROTECTIVE RESPONSE IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE.
T. D. Stein and J.A. Johnson. Pharmacy, University of Wisconsin---Madison, Madison, WI.
Alzheimer's disease (AD) is associated with increased levels of the toxic peptide, b-amyloid (Ab). This disease is modeled in Tg2576 mice by overexpression of a mutant form of human amyloid precursor protein (HuAPP). However, despite high Ab levels, plaques do not develop in these mice until an average age of 12 months, and there is no neuronal loss in the hippocampus of mice as old as 16 months. Using an Affymetrix microarray system, we identified mRNA expression levels in the hippocampus and cerebellum of three six-month-old HuAPP mice to three age-matched, transgenic controls. A ranking analysis based on Affymetrics' difference call was used to determine significant increases and decreases. RT-PCR of selected genes confirmed our results. Microarray analysis of the hippocampus demonstrated increased expression of transferrin (TFR, 29.5-fold), a protein shown to sequester Ab and prevent plaque formation, as well as a number of genes involved in neuronal proliferation, differentiation, and tissue remodeling. Of the 23 genes increased in the hippocampus, five were involved in growth factor pathways. These three genes involved in the activation of the insulin-signaling pathway were differentially regulated, including a 16.6-fold increase in insulin-like growth factor 2 (IGF2). Immunohistochemistry confirmed the hippocampal increases of IGF2 and IGFB2. The cerebellum is typically unaffected in AD and does not develop a significant age-dependent increase in Ab levels in HuAPP mice. When comparing the cerebellar gene expression in HuAPP mice to controls, the fold increase of TFR was reduced to 3.2-fold. Other genes differentially regulated in the hippocampus showed no changes at all in the cerebellum. Therefore, the slow progression and lack of full-fledged pathology in the hippocampus of HuAPP mice may result from the increased expression of several genes contributing to plaque formation.

63 OXIDATIVE DNA DAMAGE PRECEDES AMYLOID PLAQUE FORMATION IN AMYLOID PRECURSOR PROTEIN (APP)-TRANSGENIC MICE: AN ANIMAL MODEL OF ALZHEIMER'S DISEASE.
W. Jeng, M. A. Chisholm', D. Westaway' and P. G. Wells; 'Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada, 2Centre for Research in Neurodegenerative Disease, University of Toronto, Toronto, ON, Canada, and 3Department of Pharmacology, University of Toronto, Toronto, ON, Canada.
Reactive oxygen species (ROS) are implicated in neurodegenerative conditions such as Alzheimer's disease (AD), but their role in the mechanism of neurotoxicity is unclear. Here, we provide the first evidence that DNA oxidation precedes amyloid plaque formation in amyloid precursor protein (APP) mice, a transgenic mouse model of AD. To determine the relation of oxidative DNA damage, lipid peroxidation and amyloid beta (Ab) plaque deposition, a characteristic hallmark of AD, we examined the frontal cortex, hippocampus and cerebellum of APP mice, compared to age-matched wild-type controls, for endogenous DNA oxidation evidenced by 8-oxo-2-deoxyguanosine formation, and lipid peroxidation determined by thiobarbituric acid reactive substance formation. In the frontal cortex, oxidative DNA damage was enhanced within 6 weeks in APP mice, peak at 5 months (p<0.01) and returned to basal levels by 7 months. DNA oxidation was similarly elevated in hippocampal but not cerebellar tissues. In contrast, plaque formation was not observed until after 3 months of age in the frontal cortex and hippocampus, nor was lipid peroxidation elevated in any tissue at any age. These results suggest that ROS-mediated oxidation of nucleotides, as distinct from lipid, may constitute an early event in the pathogenesis of AD. (Support: Canadian Institutes of Health Research).

64 POTENTIAL ROLE OF MITOCHONDRIAL COMPLEX II ACTIVITY IN REGIONAL DIFFERENTIAL CNS SENSITIVITY TO 1,3-DINITROBENZENE-INDUCED ENCEPHALOPATHY.
A. D. Pollow, M. J. Beck and M. A. Philbert. Environmental Health Sciences, University of Michigan, Ann Arbor, MI.
Exposure to 1,3-dinitrobenzene (DNB) produces an edematous, glial-vascular lesion that is initially confined to brainstem nuclei with high energy requirements. Selective vulnerability of brainstem astrocytes to DNB is mediated by a ten-fold lower threshold for opening of the mitochondrial permeability transition (MPT) pore. Exposure of brainstem astrocytes to 100 uM DNB results in complete loss of mitochondrial membrane potential by 10 min, while their cortical counterparts remain unaffected for at least 1 hour. However, the loss of membrane potential in brainstem astrocytes is prevented by pretreatment with the known MPT pore inhibitor, cyclosporin A (CSA). DNB also decreases mitochondrial reducing potential by inhibiting FAD-linked reduction at complex II. Therefore, it is hypothesized that inhibition of mitochondrial complex II (succinate dehydrogenase) is causally linked to regional differences in susceptibility to activation of the MPT by DNB. Using cultured neonatal rat brainstem and cortical astrocytes, complex II was localized histochemically. Complex II activity is significantly inhibited in both brainstem and cortical astrocytes at 0.5, 2, and 24 hours following 1 hour exposure to 100 uM DNB. Although the observed inhibition of SDH was prevented by pretreatment with CSA in brainstem astrocytes after 0.5 and 2 hours, CSA pretreatment failed to significantly prevent inhibition of complex II activity in cortical astrocytes. These results suggest that either 1) inhibition of complex II is independent of the mechanism of DNB-induced opening of the MPT, or 2) differential regional regulation of the MPT is sufficient to account for the differential sensitivity to oxidative stress in the brainstem and cortex. However, the possibility that complex II activity is directly inhibited by DNB remains to be determined.

65 ANTI-OXIDANT PROTECTION AGAINST ETHANOLOM-INDUCED CELL LOSS IN CEREBELLAR GRANULE CELL CULTURES.
K. R. Horn, L. M. Kamendulis, C. R. Goodlett and J. L. Klaunig. Pharmacology and Toxicology, Indiana University, Indianapolis, IN; Medical Neurobiology, Indiana University, Indianapolis, IN and Psychology, IUPUI, Indianapolis, IN.
Ethanol exposure during development can cause decreases in neuronal cell populations resulting in permanent neurological deficiencies. Cerebellar granule cell cultures are vulnerable to ethanol toxicity and are useful for investigating mechanisms...
of cell death. Oxidative damage resulting from ethanol-induced increases in reactive oxygen species, have been suggested as a mechanism of ethanol-induced cell death. Recent studies utilizing antioxidants showed protection against ethanol-induced cell loss. The purpose of the present study was to investigate the possible protective effects of the antioxidants, vitamin E and Trolox against ethanol-induced cell loss in cerebellar granule cell cultures during their peak time of ethanol vulnerability (one-day in-vitro). Cerebellar granule cells were isolated from Long Evans rat pups and grown for 24 hours. Cultures were exposed to ethanol (0, 33, 166, 332 mg/dl) for 24 hours and viable cells were quantified. In addition, cultures were exposed to 332 mg/dl ethanol in the absence or presence of either vitamin E (0, 33, 100 μM) or Trolox (0, 33, 66, 100 μM) and assessed for viability. Results confirm a dose-dependent (14-29%) decrease in viable cell number following 24-hour ethanol exposure. Addition of antioxidants (vitamin E or Trolox) at concentrations of 100 μM, protected against the ethanol-induced cell loss, returning viability to that of controls (96% and 99.5%). This study suggests a role for oxidative stress, via either increased production of reactive oxygen species or decreased endogenous antioxidant levels, as a possible mechanism for ethanol-induced cell death in cerebellar granule cell cultures.

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

J.A. Johnson and J. Li. School of Pharmacy, University of Wisconsin, Madison, WI.

Increasing evidence indicates that the antioxidant response element (ARE) and Nrf2 mediate transcriptional activation of NADPH:quinone oxidoreductase and other detoxification enzymes in response to reactive oxygen species (ROS). The current study was designed to investigate the gene expression profile induced by tBHQ and determine the time-dependent gene expression patterns associated with treatment. We have analyzed gene expression induced by tBHQ (10 mM) in JMR-32 human neuroblastoma cells by means of large-scale oligonucleotide microarray. Of the 9870 genes examined, 101 showed dynamic changes with treatment from 4h to 48h. The peak of transcriptional up-regulation happened at 6h and lasted until 24h. RT-PCR for selected genes also confirmed the similar gene expression pattern. Western Blot showed changes in the corresponding encoded proteins that remained elevated for 48h. Gene cluster (SOM) analysis was used to group these genes into 12 distinct clusters with striking patterns that appear to correlate with their specific function. These included genes involved in de novo synthesis and cellular antioxidant defense, neuronal proliferation and differentiation, and signal transduction. A search for potential transcription factor binding site(s) in the 5' flanking region of the selected gene set was performed by MatInspector. The most notable observation was that genes induced by tBHQ had putative Nrf-2 binding sites, implying that a specific drug targeting effect exists. However, gene clustering analysis showed that not all of potential ARE-driven genes have the same expression profile indicating the complexity of transcriptional regulation is not dependent upon a single transcription factor or signal transduction pathway. (Funded by ES08089, ES10642, BWF New Investigator Award)

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


Pharmacology & Toxicology, Michigan State University, East Lansing, MI, Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI, National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI, and Pediatrics & Human Development, Michigan State University, East Lansing, MI.

Previous work has shown that treatment of human SVG cells with 5 μmol forskolin (F) and 250 μmol 3-isobutyl-1-methylxanthine (IBMX) increases CAMP levels resulting in differentiation and dramatic morphologic changes. We show that co-treatment with 0.5 μmol retinoic acid (RA) enhances their morphologic changes as measured by increases in the total number of processes (p<0.03) and the length of processes contacting an adjacent cell (PLP; p<0.01) over time. At 36h, RA inhibited formation of LPT (p<0.02) with increases in mean process length (p<0.04). A custom, SVG-specific cDNA microarray (2,990 genes) was used to investigate the temporal changes in gene expression during CAMP-induced differentiation. Temporal changes were identified using two different techniques. First, an incomplete block design, individual gene mixed-model ANOVA was used for analysis, followed by t-tests using a step-down Bonferroni adjustment. The mixed model included treatments, time, dye and treatment x time as fixed effects, and spon as random effects. To identify genes with significant expression changes during differentiation, least squares means of F/B/MX treated cells were compared to tissue-matched vehicle control samples utilizing t-tests. For comparison, a second technique utilizing Shannon Entropy filtering of normalized microarray data from treated, vehicle control, and untreated/time 0 control cells, with Principal Components Analysis (PCA) of the filtered data to result in the identification of expression patterns was used. Results from the two divergent approaches were comparable and identified significant temporal and treatment effects on gene expression, although some differences were observed.

BREVETOXIN EXPOSURE AUGMENTS Ca2+ OSCILLATIONS IN CEREBROCORTICAL NEURONS.

T. F. Murray, S. M. Dravid and D. G. Baden, Physiology and Pharmacology, University of Georgia, Athens, GA and Center for Marine Science Research, University of North Carolina, Wilmington, NC.

Florida red tide brevetoxins are potent sodium channel neurotoxins. Brevetoxins produce acute neuronal injury and death in cerebellar granule cells. This neurotoxic response is prevented by coactivation of nitric oxide or antagonists of the NMDA receptor. The neuroprotective efficacy of NMDA receptor antagonists has been attributed to the ability of brevetoxin to evoke the release of glutamate from cerebellar granule cells. Brevetoxins also produce a rapid increase in cytoplasmic [Ca2+]i in cerebellar granule cells with a potency consistent with that for neurotoxicity. Brevetoxin produces Ca2+ influx through three pathways: NMDA receptors, L-type Ca2+ channels and the reverse mode of operation of the Na+/Ca2+ exchanger. Oscillations in intracellular [Ca2+]i induced by neuropeptides in the plasma membrane provide critical signals to control cellular processes. To determine whether brevetoxin influence Ca2+ homeostasis generalized to neuron types besides cerebellar granule cells, we assessed the effects of brevetoxin (PbTx-2) on spontaneous Ca2+ oscillations in marine cerebrocortical neurons. This primary culture displays spontaneous oscillations in the presence of physiological concentrations of Mg2+. PbTx-2 exposure produced a concentration dependent augmentation of the Ca2+ oscillation amplitude with a potency consistent with that for activation of sodium channels. The ability of brevetoxin to affect calcium signals may affect cellular processes involved in development and learning.

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

S. M. Dravid and T. F. Murray, Physiology and Pharmacology, University of Georgia, Athens, GA.

Dextrimethorphan (DXM) is the active ingredient of commonly used antitussive preparations and represents a drug of abuse. We investigated the effect of sustained exposure of DXM and its metabolite dextrorphan (DX) on cell survival in primary cerebrocortical culture. A 48-h exposure to DX and DXM caused a concentration-dependent rise in LDH efflux and number of pyknotic nuclei as visualized by Hoechst 33258 dye staining. Other NMDA receptor antagonists were also evaluated for their propensity to produce a neurotoxic action. The rank order of potency for neurotoxicity was dizzocilpine (1α MK-801) > phencyclidine (PCP) > DX > DXM. This rank order corresponds well with the respective binding affinities of these compounds for the NMDA receptor. Coexposure of cerebrocortical neurons with DXM and noncompetitive antagonists reduced LDH efflux, further suggesting the involvement of NMDA receptors in the neurotoxicity. A 48-h exposure to NMDA receptor antagonists increased caspase-3 activity, but had no significant effect on caspase-1 activity. Intracellular Ca2+ concentrations have been found to be critical for cell survival especially during development. Real-time alterations in intracellular Ca2+ were monitored in flu-3 loaded cortical neurons exposed to MK-801 using a fluorescent plate reader (FLIPR). Acute exposure to MK-801 reduced the amplitude of calcium oscillations exhibited by neocortical neurons in a concentration dependent manner. These findings suggest that persistent exposure to DXM and DX produce toxicity in cortical neurons, which may be triggered by a decrease in intracellular Ca2+ due to the blockade of NMDA receptor. (Supported by DA 07218)

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


Neurology & Neurowurgery, McGill University, Montreal, PQ, Canada and Neurology / Neurobiology & Anatomy, University of Rochester, Rochester, NY.

There is considerable evidence that the presence of Ca2+-permeable AMPA receptors on motor neurons contributes to their vulnerability to excitotoxic injury and other insults associated with disease. Mutations in the SOD1 gene underlie some
familial cases of the motor neuron disease amyotrophic lateral sclerosis (FALS). Using a primary culture model of FALS in which G93A mutant SOD1 was expressed in motor neurons of dissociated spinal cord cultures, we demonstrated that blockade of these receptors by the specific antagonist JTX-3 prevented motor neuron death. In the present study we tested the hypothesis that reducing Ca²⁺ permeability of AMPA receptors by overexpression of Q/R edited GluR2, the glutamate receptor subunit that prevents Ca²⁺ entry through the receptor channel, would also be neuroprotective. Q/R-edited GluR2 (flo) isoform was cloned from rat cerebel lar mRNA and expressed in motor neurons of neonatal spinal cord cultures by in trans activation of plasmid expression vectors. Control vectors were "empty" vector and unedited GluR2 flo, which does not prevent Ca²⁺ influx through the receptor channel, produced by site-directed mutagenesis. Expression of GluR2 and localization to synapses was verified by double-label immunocytochemistry with anti-GluR2 and antibodies to other NR2 subunits. The transgenic mice and transgenic receptor vector died over a 9 day period. Q/R-edited GluR2 flo, but not unedited GluR2, significantly delayed motor neuron death induced by mutant SOD1. Thus, Ca²⁺ influx during neurotransmission increases vulnerability of motor neurons to toxic insult and modulation of AMPA receptor properties is a potential therapeutic strategy in ALS. Supported by MDA.

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CHARACTERIZATION OF HISTAMINE H1 RECEPTOR BINDING SITES IN FISH.

A. El-Nabawi, A. A. Choich and E. K. Silbergeig, Toxicology, University of Maryland Baltimore, Baltimore, MD.

The teleost brain is largely similar to that of higher vertebrates, but much less is known about specific neurotransmitters. In mammals, the histamine receptor has been well characterized and consists of three subtypes, H1, H2, and H3. However, in fish, little work has been carried out in identifying the presence of different subtypes of histamine receptors or on pharmacological characterization of these types. The present study was designed to pharmacologically characterize the histamine H1 receptor in the fish brain using a radioligand binding assay. Specific [3H]pyrilamine binding increased in a linear fashion, with increasing concentrations of protein over the range of 50ng to 500ng. Association of [3H]pyrilamine to the membrane was time and temperature dependent, and equilibrium was achieved after 30 min. Saturation studies revealed that the specific binding of [3H]pyrilamine to the H1 receptor was saturable, reversible, and of high affinity. Competition studies indicated that the binding was displaced by specific histamine H1 agonists and antagonists. In contrast, the selective histamine H2 and H3 receptor agonists and antagonists were very weak competitors for the [3H]pyrilamine binding sites. These results present strong evidence for the existence of the histamine H1 receptor in fish brain, and that the pharmacological profile of the H1 receptor in fish brain is apparently similar to mammalian brain. (Supported by NIEHS PP 1 P01 ES09563).

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

A. A. Choich, A. El-Nabawi and E. K. Silbergeig, Toxicology, University of Maryland Baltimore, Baltimore, MD.

Non-mammalian species, such as fish, make ideal sentinel species for supplying essential information concerning human health issues related to environmental toxicology. However, methods for identification and characterization of mechanisms of toxicity are underdeveloped. We have developed an in vivo biochemical method to evaluate neuro-active agents, according to specific regional activities within the CNS by using [14C]2-deoxyglucose (2-DG) to trace regional metabolic activity in the fish brain (Choich et al. 2001). We have used this method to characterize histaminergic pathways. Histamine is an amino acid neurotransmitter involved in arousal, attention, and sensory processing and cognition, and also plays a role in primary hormone secretion and appetite control. Structurally, the fish brain is well characterized, yet little information has been discovered about the existence and function of histamine neurotransynthesis in fish. Living, healthy fish (4 fish Tulip natalica, 10g-bw) were injected i.m. with 2uci of [14C]2-DG and allowed to swim (30min.) in freshwater with and without a histamine H1 and H2 agonist, 0.1mM (1M, 1mM, and 10mM of histamine H1 and H2 agonists, respectively). Images of histamine images were obtained using multi-modality imaging as described by Choich et al. (Toxicology Methods, Nov. 2001). The experiment was repeated in triplicate. Using digital image analysis, a clear Dose-Response in regional 2-DG uptake was evident. In the case of concentration of histamine was increased, as compared to controls), providing visual evidence of histamine H1 and H2 agonists demonstrated the applicability of 2-DG in vivo method to provide compatible and qualitative information on neuro-active agents and their direct effect on specific regions in fish brain. (Supported by NIEHS PP 1 P01 ES09563).

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IN-VIVO REACTIVITY OF THE AXONAL TOXIN 1, 2-DIACETYL BENZENE WITH NEUROFILAMENT AND MICROTUBULE PROTEINS.

M. S. Kim, M. J. Taylor, M. I. Sabir and P. S. Spencer, Center for Research on Occupational & Environmental Toxicology (CROET), Oregon Health & Science University (OHSU), Portland, OR.

Repeated treatment of young adult Sprague-Dawley rats with 1, 2-diacetylbenezene (1, 2-DAB), but not with 1, 3-diacetylbenezene (1, 3-DAB), induces irritant behavior, reduced body weight gain and evolving leg weakness with neurofilament-filled giant axons in spinal cord and associated roots. 1, 2-DAB (but not 1, 3-DAB) reacts readily in vitro with amino acids (especially l-lysine and glycine) to form a blue pigment, which is also evident in the tissues and urine of systemically treated rats. In vitro studies with purified bovine axonal proteins and rat spinal cord slices demonstrate reactivity of 1, 2-DAB (but not 1, 3-DAB) with neurofilament proteins relative to tubulin (unpublished data). Here, we report the action of 1, 2-DAB vs. 1, 3-DAB on neurofilament proteins and beta-tubulin in living nerve tissue. Surgically exposed male rat sciatic nerves were bilaterally bathed for 5 min. with saline + 2% acetic vehicle or vehicle containing 2, 5, 25 or 50mM 1, 2-DAB or 1, 3-DAB. Treated nerves were excised, desheathed and homogenized in aqueous 8M urea containing protease-inhibitor cocktail, Protein (6 microgram) from each sample was subjected to Western blot analysis. Neurofilament triplet proteins, but not tubulin, showed a concentration-dependent decrease in nerve tissue treated with 1, 2-DAB (but not 1, 3-DAB); the hierarchy of reactivity (NF-H > NF-M > NF-L) with 25mM 1, 2-DAB was comparable to that seen in spinal cord slices incubated in vivo with this chronic neurotoxin. These studies show that 1, 2-DAB preferentially targets protein components of axonal neurofilaments relative to that of microtubules. Toxicogenomic studies are underway to confirm these findings and to identify other axonal protein targets of 1, 2-DAB. Supported by NIH/NIEHS grants 1 P42 ES 10538 and 1 U19 ES11384.

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

S. V. Yeru, M. M. Iban and J. V. Marino, Biology Department, Rutgers University, Camden, NJ and Department of Pharmacology and Toxicology, EOHSI, Rutgers University, Piscataway, NJ.

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

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

D. W. Siddles, Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA.

Changes in nodal Na and K channel quantity and distribution by acrylamide (ACR) or 2, 5-hexanediene (2, 5-HDE) were evaluated as potential mechanisms in the production of neurotoxic symptoms. Sprague-Dawley rats (25-250g) were given either high (50mg/kg i.p. for 9 days) or low (2.6mg/kg drinking water for 49d) dose ACR or high (40mg/kg) and low dose 2, 5-HDE, until adult littermates served as controls. At the endpoints, animals were acetic and demonstrated significant hindlimb paralysis, particularly both ACR groups. Fixed tibial nerves were gently teased to single fibers and processed for identification of Na (monoclonal anti-sodium, clone K38/55, Sigma #S8809) and K (rabbit

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for evaluation of toxicant-induced changes in neural function and antiepileptic drug (AED) treatments.

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

J. F. Ross¹, E. J. Lehning² and B. M. LoPachin³
¹The Health & Environmental Safety Alliance, 442 Oliver Rd, Cincinnati, OH and ²Anatomiya Research, Montefiore Medical Center, Bronx, NY.

The influence of dose-rate on spatiotemporal characteristics of neurodegeneration in CNS of ACR intoxicated rats has not been well documented. Therefore, animals were exposed to daily ACR at either 50 mg/kg (p.o.) or 21 mg/kg (p.o.) and brains and spinal cords were processed using de Olmos' amino capric silver stain for detecting degenerating nerve cell bodies, dendrites, axons and terminals. For each dose-rate, experimental times were selected according to the development of ACR-induced neurological defects; i.e., ataxia and hindlimb foot-splay. Results show that, in spinal cord, ACR intoxication at either dose-rate produced nerve terminal degeneration in several gray matter regions (e.g., layers 5-7 of intermediate zone, lemniscus, and intermediolateral cell column). However, axon degeneration in spinal white matter was related to dose-rate; i.e., the lower ACR dose rate (21 mg/kg/d) was associated with significant axon degeneration primarily in the gracile fasciculus and pyramidal tract, whereas during higher dose-rate exposure axon involvement was rare. In cerebellum, selective Purkinje cell damage was evident regardless of dose-rate. This effect was characterized by early degeneration of Purkinje cell dendrites in the molecular layer and nerve terminals in cerebellar and vestibular nuclei. The onset of axon angiophila in cerebellar white matter was relatively delayed. In hindbrain, both dose-rates produced predominant nerve terminal degeneration in pretectal nuclear regions (e.g., lateral reticular nucleus), gracile and cuneate nuclei and certain nuclei of the reticular formation (e.g., pontine nucleus). Neither dose-rate caused axon degeneration in hindbrain white matter. Thus, nerve terminal degeneration in the CNS is a consistent effect of ACR across dose-rates, whereas axon degeneration is either delayed or occurs only during low dose intoxication. This supports the hypothesis that the nerve terminal is a primary site of ACR action. Sponsored by NIH grant ES03980-16

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

R. M. LoPachin, S. Manukhan and S. Das. Anesthesia Research, Montefiore Medical Center, Bronx, NY.

We hypothesize that the nerve terminal is the primary site of ACR action and that binding of this tricarboxylic acid containing synaptosomal protein disrupts presynaptic structure and function. As an initial investigation of molecular mechanisms, we characterized the binding of ACR to synaptosomal proteins. Rat cerebral cortex synaptosomes (250 µg protein) were prepared on Percoll gradients and were incubated in oxygenated medium for 1 hr with 14C-ACR (7 µCi; 5 mCi/mmol). Labeled synaptosomal proteins were resolved by gel electrophoresis and then trans-
ferred to nitrocellulose membranes. Protein bound radiolabel was visualized on autoradiographs formed by exposing membranes to X-ray film for 21 days. Comparison of comassie blue-stained gels with autoradiograms revealed that among numerous potential targets, six ACR-labeled proteins could be identified. The estimated molecular weights of several unknown proteins matched that of specific SNARE proteins: e.g., SNAP-25 (25 kDa), syntaxin (38 kDa) and synaptogamin (58 kDa). Radiolabelling of proteins could be completely prevented by preincubation with the sulphhydryl reagent N-ethylmaleimide (NEM, 4 mM), but not by exposure to propionic anhydride (10 mM) or to the monothiol-reducing agent, dithiothreitol (4 mM). Determination of the relative sulphhydryl reactivities of ACR, NEM and iodoacetamide (IA) showed that these chemicals were equally efficacious with respect to reducing DTNB-determined non enzymatic -SH content, although the order of potency differed; NEM > IA > ACR. These data suggest that ACR forms sulphhydryl adducts with specific proteins and provide initial support for our hypothesis that ACR neurotoxicity is mediated by nerve terminal sites of action. Sponsored by NIH grant ES03830-16.

81 ELECTRON MICROSCOPIC EVALUATION OF THE CEREBRAL CORTEX IN ACYLAMIDE EXPOSED RATS.

Male Sprague-Dawley rats (250-275 g) were administered 50 mg/kg acylamide intraperitoneally for 5 or 11 days, and then sacrificed for ultrastructural examination of the cerebral cortex. Light microscopy revealed no changes in the molecular layers. Beginning on day 5, transmission electron microscopic study of the cortex demonstrated a selective effect on the Purkinje cells. Some nuclei were eccentric in location. Their perikaryal cytoplasm contained disordered and diminished Nissl substance, particularly in the rough endoplasmic reticulum component, and increase in tubular and vesicular profiles. Occasional large double membrane-lined vacuoles, likely representing swollen astrocytic intrusions into the altered perikaryon, were noted. Swollen Purkinje cell dendrites containing electron dense aggregates of degraded organelles were present in the molecular layer. Altered mitochondria were a prominent feature of these degraded masses, which sometimes filled post-synaptic regions of the dendrites. Ascent astrocytes were swollen. High degenerating myelinated axons were seen in the granular layer. These findings indicate that the Purkinje cell is a cerebellar target during acylamide intoxication, and confirm in part the findings of an earlier electron microscopic study (Cavagnan and Gysbers, J. Neurocytol. 12:413-437, 1983). We also demonstrate a cellular and ultrastructural basis for our companion silver impregnation study of degenerating cerebellar Purkinje cells (Ross et al., Abstract of the 2002 Society of Toxicology meeting). Supported by NIH grant ES03830-16.

82 N,N-DIETHYLDITHIOCARBAMATE, BUT NOT S-METHYL-N,N-DIETHYLDITHIOCARBAMATE, PRODUCES A DISULFIRAM-LIKE SCHWANNOPATHY IN RATS.
E. G. Tunkin, H. L. Valentine and W. M. Valentine. Pathology, Vanderbilt University Medical Center, Nashville, TN.

Disulfiram, a dihydrocarbamate drug used in alcohol aversion therapy, produces a peripheral neuropathy that was characterized in rats at a segmental demyelination accompanying generation of S-(N,N-diethylthiocarbonyl) cysteine (DET-Cys) adducts. To assess the role of cystine carboxylation by the 2-disulfiram monooxygenase N,N-diethyldithiocarbamate (DEDAC) and S-methyl-N,N-diethyldithiocarbamate (MeDEDAC), were administered parenterally to male Sprague-Dawley rats via osmotic infusion pumps for 4 and 8 weeks. Following exposure, hemoglobin and spinal cord axial proteins were isolated and analyzed for the presence of DET-Cys adducts, while peripheral nerve and spinal cord sections were obtained and examined by light and electron microscopy for the presence of morphological lesions. Globin preparations from both DEDAC- and MeDEDAC-exposed animals were found by RP-HPLC to contain a late-eluting β-globin peak, the quantity of which increased as a function of exposure level and duration, consistent with the formation of a DET-Cys adduct on the B β-chain face of the protein. These data suggest that formation of DET-Cys-protein adducts was verified after hydrolysis of both globin and spinal cord axial proteins by LC-MS/MS analysis. The quantity of DET-Cys adducts produced on hemoglobin and axial proteins was two fold greater for MeDEDAC relative to DEDAC. Demyelinated axons, myelin edema, and other Schwann cell lesions similar to those seen with disulfiram treatment, were observed in all peripheral nerves examined from animals treated with 0.25 or 0.35 mmol/kg DEDAC for 8 weeks. No lesions were detected following exposure to MeDEDAC at any time point or dose level. These results indicate that peripheral administration of DEDAC produces a neuropathy similar to disulfiram, but the histopathological nerve lesions following MeDEDAC treatment, were observed in all peripheral nerves examined from animals treated with 0.25 or 0.35 mmol/kg DEDAC for 8 weeks.

83 PROTECTION AGAINST METHANOL-INDUCED RETINAL TOXICITY BY LED PHOTOSTIMULATION.
J. T. Ellis, M. M. Henry, P. Summerfeld, M. T. Wong-Riley, E. V. Bachmann and H. T. Whelan. Medical College of Wisconsin, Milwaukee, WI.

Decrement in mitochondrial function have been postulated to be involved in the pathogenesis of macular degeneration, diabetic retinopathy, Leber's hereditary optic neuropathy and methanol intoxication. Formic acid is the toxic metabolite responsible for the retinal and optic nerve toxicity produced in methanol intoxication. Previous studies in our laboratory have documented formate-induced mitochondrial dysfunction and retinal toxicity in a rodent model of methanol intoxication. Photostimulation by monochromatic red to near-infrared radiation has been postulated to enhance mitochondrial activity by stimulation of cytochrome oxidase. The present studies were undertaken to test the hypothesis that exposure to 670 nm light from light-emitting diode (LED) arrays would protect the retina against the mitotoxic and cytotoxic actions of formate. One group of methanol intoxicated animals was intoxicated with methanol in the absence of LED treatment and a second group was intoxicated with methanol and exposed to LED treatment (670 nm for 1 min 45 seconds - 50 mW/cm², 4 joules/cm²) at 5, 25, and 50 hours after the initial dose of methanol. Blood formate concentrations did not differ between the two treatment groups and averaged 5.6 ± 1.0 mM at 72 hours. At 72 hours of methanol intoxication, retinal function was assessed by measurement of ERG responses and retinas were prepared for biochemical and histologic analysis. ERG responses recorded in methanol-intoxicated animals revealed profound attenuation of both rod-dominated and UV-mediated responses. In contrast, methanol-intoxicated animals exposed to LED treatment exhibited a complete recovery of rod-dominated ERG responses and partial recovery of UV-mediated ERG responses. These data provide evidence that LED phototherapy protects the retina against the cytotoxic actions of formate and are consistent with the hypothesis that LED photostimulation improves mitochondrial respiratory chain function. (Supported by NIH RO1 ES06648, RO1-EY11396, P30-EY01931 and NASA N89-90015).

84 THE INDUCTION OF AMYLOID PRECURSOR PROTEIN AND α-SYNUCLEIN IN ASTROGLIA BY DIETHYLDITHIOCARBAMATE (DEDAC) AND COPPER WITH OR WITHOUT GLUTATHIONE (GSH).
S. Cheng and L. D. Trombetta. Pharmaceutical Sciences, St. John University, Jamaica, NY.

α-synuclein has been hypothesized to provide a nucleation centre during the formation of senile plaques. It has been shown that α-synuclein undergoes self-oligomerization when β-amyloid (AB) is present. Gli cultures produce the Kunitz-protease inhibitor (KPI) - containing APP. The KPI-containing APP protein may be implicated in Alzheimer's disease (AD) pathogenesis. Astroglia may play a role in the pathogenesis of AD. The role of xenobiotics in the pathogenesis of neurodegenerative disease has been suggested. The mechanism of DDC neurotoxicity is thought to involve Cu²⁺ chelation and to interact with SH group to destroy the GSH homoeostasis and inhibit sulphuric enzymes. GSH has been shown to protect against DDC cytotoxicity. Cu has been shown to induce the self-oligomerization of α-synuclein. APP contains a Cu-binding site. APP reduces bound Cu²⁺ to Cu⁺, and could induce the formation of hydroxy radicals. In our study in astroglia were grown in complete medium in standard culture plates at 37°C. 775 flask. Astroglia were treated with DDC 17.5 μg/ml, Cu 0.2 μM, or DDC 17.5 μg/ml + Cu 0.2 μM in medium for 1 hr. Cells were washed in PBS and refed with complete medium with or without 10 μM GSH. Immunocytochemistry was performed at 4 and 8 hr post treatment using APP or α-synuclein primary antibody followed by gold conjugated secondary antibody. The presence of APP and α-synuclein protein production occurring in a time-dependent manner. At 4 hr post treatment, the cells contained small positively stained material deposited throughout the cytosol. This material increased in size and density over time, and by 8 hr it appeared as a dense aggregate surrounding the nucleus. Western Blots were performed at 4 and 8 hr post treatment using APP or α-synuclein primary antibody followed by horseradish peroxidase-linked secondary antibody. Immunoblot supported immunocytochemistry results. GSH showed significant protection at 8 hr post treatment.

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

We have previously demonstrated that the PCB mixture, Aroclor 1254 (A1254), increases the phosphorylated form of CREB (pCREB), the GMP-responsive element binding protein. This transcription factor is important in nervous system development and plasticity. Phosphorylation of CREB results in its activation and is
mediated by a variety of stimuli, including activation of protein kinases A and C and increases in intracellular calcium. As such, the present experiments were designed to determine the mechanisms through which PCBs increase pCREB. Using digital microscopy, semiquantitative fluorescent immunocytochemistry of sections of phosphorylated CREB was performed in primary cultures of cortical neurons in the presence and absence of A21254 and agonists and antagonists of PKA, PKC, intracellular Ca2+. Cellular pCREB fluorescence intensity was analyzed with the aid of image analysis software. The pCREB responses were expressed as Gaussian distributions over the half-maximal fluorescence (EC50) of the population was determined by computer analysis. In controls, pCREB was constitutively and non-uniformly expressed. A21254 (20 μM) increased pCREB immunoreactivity in a dose-dependent manner. A21254 (10 μM) or the agonists glutamate, forskolin and phorbol myristate acetate produced EC50 values for pCREB fluorescence by 36±5, 51±15, 47±9 and 40±10%, respectively. PKA inhibitor peptide (40 min preincubation) completely inhibited, whereas BAPTA-AM or tetrodotoxin partially inhibited, and the PKC inhibitor, bisindolylmaleimide, did not inhibit pCREB induction by A21254 (10 μM, 40 min). We also examined whether cAMP is involved in the activation of pCREB by PKC. To adenylate cyclase activator, forskolin, but not A21254 (20 μM) elevated intracellular Ca2+ levels. These data support a role of a PKA and intracellular Ca2+ pathways involved in PCB-induced CREB phosphorylation. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

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

W. R. Mundy, J. R. Inglefield and T. J. Shaffer, Neurosciences Division, NHEERL, ORD, USEPA, Research Triangle Park, NC.

Alteration of calcium signaling has been proposed as a possible mechanism contributing to the developmental neurotoxicity of polychlorinated biphenyls (PCBs). Little is known, however, regarding the actions of structurally related compounds such as polychlorinated diphenyl ethers (PBDEs), flame retardants whose levels are rising rapidly in the environment. We examined the actions of the PCB mixture Aroclor 1254 (A21254), which is known to alter calcium homeostasis, and a PBDE mixture (DE71), on activation of the mitogen-activated protein kinase (MAPK) cascade. MAPK is a critical regulator of cell division and differentiation which can be activated by numerous signals including increases in intracellular calcium. Immature primary cultures of rat cortical neurons were deprived of serum overnight and exposed to A21245, DE71, or the agonists glutamate and KCI. Activation of MAPK was assessed by measuring the dual phosphorylated, active form of MAPK by SDS-PAGE and immunoblotting with a phospho-specific antibody. In immature cortical neurons, activation of ionotropic and metabotropic glutamate receptors with glutamate resulted in a time- and concentration-dependent increase in phopho-MAPK. This activation could be partially blocked using calcium-free buffer in the presence of the calcium chelator BAPTA. Similar effects on MAPK activation were observed after depolarization of cortical neurons with KCI. Stimulation with both A21254 and DE71 (1-30 μM) resulted in a time- and concentration-dependent increase in phospho-MAPK. A21254 was more potent than DE71, and the effects were partially blocked in the absence of calcium. These results suggest that PCBs and the structurally related PBDEs can affect the MAPK kinase cascade, a critical mediator of cell development and neuronal plasticity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

85 QUANTIFICATION OF CYP GENE EXPRESSION DETERMINED BY LIGHTCYCLER TECHNOLOGY.


Changes in drug-metabolizing enzymes sometimes alter toxicological consequences. Although drug-metabolizing enzymes, especially cytochrome P450 (CYP) levels, after exposure of chemicals are generally assessed by expressing protein levels using western blotting, it is considered that quantification of their gene expression levels provides equivalent or more valuable information. However, the combined method of RT-PCR and Northern blotting, which has been generally used, is time consuming. We have achieved quantification of CYP gene expression levels by real-time PCR (LightCycler17), Roche). RNA was isolated from rat liver and reverse transcription was conducted. Gene expression was quantified by the real-time PCR method consisting of PCR in a capillary tube and online monitoring of fluorescence of SYBR Green I, which is specifically bound to stranded DNA produced by PCR reaction. Using this method CYP induction after administration of deoxymethanex (DEX) and 3-methylcholanthrene (3-MC), well known enzyme inducers, were examined. DEX (60 mg/kg) or 3-MC (50 mg/kg) was given by an i.p. injection to female SD rats, and liver samples were collected 24 hours later. Primers corresponding to CYP molecular species (CYP1A2, 2B1, 2B2, 3A2, 3A9, 3A23) and β-actin were used and all the molecular species examined were quantifiable. Significant enzyme induction was observed in CYP2B3 and 1A12 in the DEX treated group and in CYP1A2 in the 3-MC treated group. The advantages of this method are that analysis time can be reduced to less than 60 minutes without risk of contamination and use of labor-intensive gel electrophoresis, and that multiple samples (maximally 32 samples containing controls) can be examined simultaneously. These results suggest that real-time PCR by LightCycler is a useful tool for rapid determination of altered CYP gene expression and possibly other target genes induced by chemical exposure.

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

A. Kachroo and J. E. Chambers, CEHS, Mississippi State University, Mississippi State, MS.

Phosphorodihionate insecticides can be activated to their oxon metabolites by deamination or detoxification by deamination, both reactions are mediated by P450's. The effect of gestational/lactational exposure to PCB's (polychlorinated biphenyls) on hepatic microsomal metabolism of the organophosphorus insecticides diazinon, azinphosmethyl, dimethoate, and coumaphos was determined. The dams were administered the PCB mixture Aroclor 1254 from GD36 to PND 22. The potential of detoxication, deamination, and diazinon was monitored using HPLC methodology in naive male rats (PND 3, 12, 21), and in male and female rats exposed to PCB's (PND 15 and 23). Data showed a general increase in pyrimidinyl (diazinon deamination) production with age, with 70d and 33d statistically different from one another, whereas, the other ages 3, 12, 21 which were not statistically different from one another. PCB treated rats showed a statistically significant increase in pyrimidinyl production at all ages and in both sexes. Analysis of coumaphos phenol production showed a general age-related increase with no statistical difference between the naive rat group; however data in PCB treated animals showed a similar trend as with diazinon deamination. Diazinon, dimethoate, and coumaphos desulfituration potentials (measured indirectly acetylene incorporation inhibition) showed no significant differences among ages, but did exhibit a general age-related increase in naive rats. Data for azinphosmethyl activation exhibited an age-related increase with 70d and 33d not statistically different from one another, but different for the rest of the ages studied. PCB treated rats showed significantly greater desulfituration for diazinon, azinphosmethyl, and coumaphos at all ages and with both sexes. Dimethoate desulfituration showed no statistical difference in the PCB treated groups. The data indicate that age-related differences in desulfituration/deamination potential for naive rats is compound specific as the ability to be induced by PCB exposure (EPA R 82596-01).

87 IN VITRO METABOLISM OF OCTAMETHYLCYCLOTRISILANOLXANE BY HUMAN AND RAT MICROSONES.

F. A. Dias, E. Usuki, J. M. McKim Jr., A. Madam, A. Parkinson and K. H. Plashi, Dow Corning Corporation, Midland, MI and Nvotion LLC, Kansas City, KS.

Exposure of rats to Octamethylcycloooxane (D4) induces CYP450 enzymes in the liver, and causes hepatomegaly and transient increases in hepatocyte proliferation similar to phenobarbital (PB). It also appears that these enzymes are capable of recognizing D4 as a substrate and may play a role in its metabolism and elimination in the rat. This study was designed to determine if D4 is metabolized by liver microsomes from humans or rats and to identify which specific CYP450s are responsible for the metabolism in humans. 14C-D4 was incubated with recombinant (r) human CYP450s and liver microsomes (rat & human) at 37°C for up to 2 hr and at various protein concentrations. Incubation extracts were analyzed by HPLC with radiometric detection. Chromatograms from PB-rat incubations contained at least 8 distinct peaks (metabolites M1-M8) whereas only 2 metabolites (M5, M8) were detected in saline-rat incubations. Human microsomes gave rise to predominately 1 metabolite (M8) with M5 detected only at high protein concentrations. Of the CYP450s evaluated, only CYP2B6 (M5 & M8) and CYP3A4 (M8) produced metabolites. Formation of M8 and/or M5 by human microsomes and CYP3A4 was not proportional to time or protein concentration. Formation of M8 by CYP2B6 was proportional to protein concentration but not time. M8 formation in saline-rat incubations was proportional to protein concentration but not time. In PB-rat incubations a few metabolites showed proportionality between formation and incubation time and/or protein concentration, however M8 did not. These experiments revealed that rats and human liver microsomes are capable of me-
tabulating D4 and PB induction in rats greatly enhances the ability of liver microsomes to metabolize D4 to a variety of metabolites. The use of CYP450s and inhibitory polyclonal antibodies demonstrated that human CYP2B6 and CYP3A4 are likely involved in the observed metabolism. Supported in part by the National Institute of Environmental Health and Safety, U.S. Environmental Protection Agency, and the Environmental Protection Agency, U.S. Environmental Protection Agency.

88 PRECISION-CUT HUMAN LIVER SLICES AS A MODEL FOR EXAMINING PHARMACOGENETIC INTERACTIONS: CYTOCHROME P450 EXPRESSION AND SENSITIVITY TO DRUG-DRUG INTERACTIONS.

H. J. Eindol, S. Bedi-Singh and V. Fischer. Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Precision-cut human liver slices offers a practical alternative to hepatocyte cell cultures to examine cytochrome P450 (CYP) induction by compounds that predict or preclude potential drug-drug interactions. Induction of P50 gene expression was measured in human liver slices by real-time PCR using probes/primers developed to specifically recognize CYP1A1, CYP1A2, CYP3A4, CYP2C8, CYP2C9, or CYP2C18 mRNA. Liver slice viability was measured by monitoring of ATP; GSH, and K+ content. Minimal loss in slice viability occurred between 24 h and 48 h (1.2- and 1.7-fold decreases in K+ and ATP content, respectively), whereas, more significant losses of viability occurred after 72 h. CYP1A1 and CYP1A2 gene expression was induced by β-naphthoflavone (50 μM), with respect to vehicle control, 39- and 110-fold at 24 h of treatment and 19- and 55-fold at 48 h, respectively, which indicated that maximal induction occurred at or prior to 24 h. Inducers of CYP3A4, carmamazine (CAR, 50 μM), rifampin (RIF, 50 μM), phenobarbital (PB, 0.5 mM), and desoximethasone (DEX, 50 μM) induced CYP3A4 gene expression 2.4-, 4.6-, 7.6- and 3.6-fold at 24 h, and 2.9-, 7.1-, 18-, and 10-fold at 48 h, respectively. Known inducers of CYP2C enzymes, Rif, PB, and DEX increased CYP2C mRNA expression by 1.5-, 3.1-, and 2.3-fold at 24 h and by 1.3-, 3.9-, and 3.5-fold at 48 h, respectively; RIF, PB, and DEX induced CYP2B mRNA expression by 6.0-, 7.0-, and 7.4-fold at 24 h, and 2.7- and 4.6-fold at 48 h, respectively. DEX induced CYP1A2 mRNA by 48 h (2.4-fold), whereas PB and Rif had little effect. The levels of induction of CYP3A4 and CYP3A4 mRNA were similar to induction levels found recently in human hepatocytes. These studies indicate that induction of P450 gene expression can be measured in human liver slices with a single treatment of inducer within 24 h of slice culturing and up to 48 h with minimal reduction in slice viability.

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


Cytochrome P450 induction in liver by a variety of enzyme inducing chemicals demonstrates switch-like behaviors. As dose increases, more cells become fully induced rather than observing a proportionate induction in all cells in the liver. These inducers interact with different receptor systems, indicating that this switch-like response is a generic property of induction with these cells. The molecular mechanisms that control these switches remain unknown. We have developed an in vitro model system using cultured rat hepatocytes to study this phenomenon with prototypical inducers. In this study we examined induction of CYP1A1 protein and mRNA by PCB 126, a prototypical inducer of hydroxysteroid receptor (SXR) agonist. Hepatocytes from male Sprague-Dawley rats were treated for 24 hours after attachment on plates at 1250 concentrations ranging from 10 to 1000 μM. Total CYP1A1 protein was evaluated in cell homogenates by densitometry of Western blots and for CYP1A1 mRNA by quantitative PCR. The EC50s for induction of both protein and mRNA were in the range of 2 to 5 μM. These integrated responses of populations of hepatocytes were compared with responses of individual cells by visualization of treated cells on plates. Protein was visualized by CYP1A1 immunohistochemistry. While induction of individual cells and the Dose-Response for increasing numbers of cells stained with increasing dose was evident by immunohistochemistry, further refinement of imaging techniques are necessary to derive quantitative estimates of numbers of cells induced at each dose level. Nonetheless, the combined evaluation of population and cell level responses of hepatocytes offered by this in vitro model permits characterization of the induction switch and provides a system to probe the molecular mechanisms that underlie the non-linear dose-response behaviors for these receptor-mediated toxicants. This work was supported by a contract from the American Chemistry Council.

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

S. E. Boldt and D. E. Amacher. Molecular and Investigative Toxicology, Genentech Laboratories, Pfizer Global Research and Development, Groton, CT.

Many xenobiotics are more toxic when metabolized by cytochrome P450 enzymes (CYPs). The intent of this study was to test such chemicals in Gentest® cell lines, which are human lymphoblasts that have undergone stable transfections resulting in expression of human CYPs. Aflatoxin B1 (A7) was tested in the cell lines hA2/2, hA3/4, hE2/1OR, hE2/1OR, 292-Agro/2, 2D6-VaL, MCL-5 (CYP1A2, 3A4, 2E1, 2A6, and elevated native 1A1), and the control lines hE0 and hE6 (no CYP over-expression) for 72 hours alone and concurrently with inhibitors. Inhibitors included as follows: 5 μM furafylline (3A4), 10 μM ketoconazole (3A4), 10 μM sulfaphenazole (2C3), 10 μM 6-4-hydroxyrezone (2E1), 10 μM quinidine (2D6), and 15 μM amilorinbrentozone (ABT, all). Furafylline inhibited CYP-mediated ABT toxicity completely in the hA2/2 cells but not in the MCL-5 cells. Ketoconazole inhibited ABT toxicity completely in the hA3/4/4 cells but not in the MCL-5 cells. Co-treatment with ABT in the MCL-5, hA2/2, and hA3/4/4 cell lines did not inhibit toxicity. ABT did not demonstrate toxicity in the other cell lines. Aflatoxin B2, B1a, and G2 were not toxic to any of the cell lines at the concentrations tested. Aflatoxin G1 (AG1) and Aflatoxin G2 (AG2) displayed CYP-mediated toxicity similar to that of ABT. Aflatoxin M1 (AM1) was toxic only to the hA2/2 and MCL-5 cell lines when tested up to 0.5 μM, while Aflatoxin M2 did not exhibit toxicity in any of the cell lines when tested up to 0.25 μM. Cellobichine, which is directly toxic, was used as a positive control in these assays. In conclusion, based on results with these cell lines, ABT, AG1, and AG2 toxicity are CYP-mediated, and are mediated principally by CYP1A2 and to a lesser extent by CYP3A4.

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

M. Ghanem, F. B. Bault, P. K. Unlu, V. Vallarath, J. Y. Ma, M. Burger, J. Nauth, V. Castranova, and A. F. Hubbs. Health Effects Laboratory Division, NIOSH, CDC, Morgantown, WV and West Virginia University, Morgantown, WV.

Cytochrome P450 inhibitors are an important pharmacological tool in human and rat models. However, their utility is limited by the induction of other cytochromes, which may alter the results. In this study, we examined the effect of coal dust on pulmonary cytokine induction in rats. Rats were exposed to coal dust for 6 weeks and the pulmonary cytokine levels were measured. The results showed that coal dust significantly increased the levels of IL-1β and TNF-α. The inhibition of CYP1A1 and CYP1B1 activity by coal dust was assessed using the 7-ethylbenz[a]pyrene hydroxylase and 7-ethoxycoumarin O-dealkylase assays. The results showed that coal dust significantly increased the levels of IL-1β and TNF-α. The inhibition of CYP1A1 activity by coal dust was assessed using the 7-ethylbenz[a]pyrene hydroxylase and 7-ethoxycoumarin O-dealkylase assays. The results showed that coal dust significantly increased the levels of IL-1β and TNF-α.
cells. Co-treatment of the cells with 2 μM α-naphthoflavone, a P450 inhibitor and arylhydrocarbon receptor antagonist, blocked the increase of BENZO(A)PYRENE hydroxylase activity by treatment with MEP extract alone. Immunoblot analyses of 59 proteins using a mouse monoclonal antibody 1-12-5 against the rat P450 1A1 and a rabbit polyclonal antibody against human P450 1B1 revealed that MEP extract induced proteins immunorelated to P450s 1A1 and 1B1. RNA blot analysis of total RNA using a human P450 1A1 3' end and a human P450 1B1 RT-PCR product cDNA probes showed that MEP extract increased the levels of P450s 1A1 and 1B1 mRNA hybridizable to the respective mRNA probes. Treatment with 10 μM BENZO(A)PYRENE, a component of MEP extract, for 24h induced cat- alytic activity, protein, and mRNA of P450s 1A1 and 1B1 in MCF-7 cells. Treatment with MEP extract increased P450s 1A1 and 1B1 proteins and mRNA levels in NCI-H2292 human lung carcinoma and A549 human lung adenocarcinoma cells. The extract also increased P450 1A1, but not P450 1B1, protein and mRNA in HepG2 human hepatoma cells. The present findings demonstrate that MEP extract has the ability to induce P450s 1A1 and 1B1 in the estrogen-responsive MCF-7 cells. Induction of the carcinogen- and estrogen-metabolizing P450s 1A1 and 1B1 may be an important factor to consider in assessing the potential health effects associated with human exposure to ME.

**REGIONAL INDUCTION OF CYP1A1 PROTEIN DURING TREATMENT OF RATS WITH MIXTURES OF PCB126 AND PCB153.**
Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Colorado State University, Fort Collins, CO.

Liver enzyme induction by various inducers is regional and has all-or-none, switch-like characteristics in vivo and in vitro. We have evaluated enzyme induction and liver tumor promotion of a mixture of planar (PCB126) and non-planar (PCB153) PCBs in Fisher rats using a modified fry initiation-promotion study. Starting two weeks after Fry Initiation, rats were injected 3 times per week with PCBs. In a second study, enzyme activity for CYP1A1 was estimated in liver micro- somes from Sprague-Dawley rats treated with PCBs for 13 to 30 weeks. In the Fry study, concentrations of PCBs in liver were determined for rats treated with mixes or with either compound alone. At moderate doses, there was a more than additive increase in enzyme activity in the multi-week dosing study and an increased liver concentration of PCB153 in mixture treated rats in the Fry study. At moderate to high doses, there were less than additive increases in number and area of GST P1-foci. In the Fry study, exposure to PCB126 caused regional induction of CYP1A1, starting in the centrilobular region at 0.1 μg/kg/day and progressively outward until all the liver was induced at 10 μg/kg/day. In the high dose PCB126 rats that also received a high dose of PCB153 (10, 600 μg/kg/day), the PCB153 concentrations reached 76 μg/g liver, 3-fold higher than those treated with PCB153 alone. In the mixture treated rats, the pattern of induction changed. Instead of complete induction of CYP1A1 throughout the liver as in the high dose PCB 126 rats, the pattern was regional showing preferential periplural staining, a reversal of the normal cen- trilobular pattern. This unexpected regional induction may correlate with the inter- nary interactions for GST-P1 foci. This will also be studied in an in vivo switching model (Ito French et al., this meeting). This work was supported by contract #467 from the American Chemistry Council and NIH grants SBIR 525994, KOB ES03314 and KOB ES03389.

**94 INFLUENCE OF SIGNAL TRANSDUCTION PATHWAYS ON TCDD-MEDIATED CYP1A1 GENE EXPRESSION.**
D. E. Machemer* and R. H. Tuley*.
*Department of Pharmacology, University of California, Davis, CA and Department of Chemistry & Biochemistry, University of California, Davis, CA.

Cytochrome P450 1A1 (CYP1A1) catalyzes the hydroxylation of a variety of xenobiotic compounds and is strongly induced by halogenated and polycyclic aromatic hydrocarbons. Protein kinase C (PKC) has been implicated in the regulation of this response, since 1 several PKC inhibitors have been shown to inhibit induction by Ah receptor ligands, and 2) phorbol esters are synergistic to TCDD-mediated induction of CYP1A1. To define the role of PKC in regulation of the CYP1A1 gene, we examined the effect of specific PKC inhibitors on this response. Experiments were conducted using the human HepG2 TV101 cell line, which stably express the firefly luciferase gene under control of the CYP1A1 promoter (+1612 bp). TV101 cells were treated with 5 nm TCDD for 6 hours in the presence or absence of staurosporine inhibition of several specific PKC inhibitors (G109293SX, G06993, and G06976). TCDD alone did not significantly increase PKC activity. Phorbol 12-myristate 13-acetate (PMA) caused a 2- to 5-fold increase in luciferase activity both in the absence of TCDD (untreated compared to PMA treated TV101 cells) and the presence of TCDD (TCDD treated compared to TCDD and PMA co-treated TV101 cells). TV101 cells co-treated with TCDD and staurosporine exhibited a dose-dependent inhibition of PKC activity and of CYP1A1- luciferase activity at similar staurosporine concentrations. However, TV101 cells co-treated with TCDD and a specific PKC inhibitor showed a difference of approximately one order of magnitude between inhibitor concentrations necessary for dose-dependent inhibition of PKC activity and inhibitor concentrations necessary for dose-dependent inhibition of CYP1A1-luciferase activity. The magnitude of this difference was the same for TV101 cells co-treated with TCDD, PMA, and each of the specific PKC inhibitors. Combined, these results suggest that the actions of PKC may not constitute the sole component of CYP1A1 regulation. (Supported in part by URSK grant ES03373).

**95 HIGHER STABILITY OF TCDD-REGULATED CYP1A1 AND CYP1B1 mRNA IN THE MURINE AH RECEPTOR-DEFICIENT HEPA VARIANT THAN IN THE WILD TYPE HEPA-1C1C7 CELL LINE.**
S. E. Eiler and C. J. Feige.
*Pharmacology, Merck, Medical College, Nashville, TN and *Pharmacology, University of Wisconsin, Madison, WI.

The induction of CYP1A1 and CYP1B1 mRNA by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in Hepa-1 cells, wild type (WT) and the low Ah receptor (AhR) level variant (LA1) was examined. The kinetic analysis of the induction showed major differences related to the stability of the AhR and its two regulated genes. TCDD treatment of Hepa cells results in increased transcription of CYP1A1 and CYP1B1 with a subsequent down regulation of the AhR. We have shown that nuclear translocation of the receptor is a pre-quistic for this decrease and that simultaneous treatment with actinomycin D reversed the TCDD-activated loss of AhR in WT. This indicates that continuous transcription is necessary to maintain receptor degradation by an apparently stable cytoplasmic receptor in LA1, however AhR proteolysis was high in absence of TCDD treatment, and actinomycin D treatment stabilized the cytosolic AhR levels only in LA1 cells, suggesting that the transcription-sensitive AhR degradation in LA1 cells is cytosolic rather than nuclear and might have contributed to the 10-fold lower constitutive AhR levels in LA1 variant. The transcription of CYP1A1 and CYP1B1 mRNAs by TCDD in WT cells each exhibited bursts of activity in the initial hour which would have lasted longer than in LA1 cells, approximately in proportion to the respective nuclear AhR levels after 1 h. However, the induced mRNA levels in LA1 exhibited a slow and pro- longed increase reaching 50-500 percent of the WT steady state levels by 20 h. The decay of both CYP1A1 and CYP1B1 mRNAs followed inhibition of transcription by actinomycin D was much slower in LA1 cells. This slower mRNA turnover therefore, compensates for lower initial transcription rate and allows LA1 to gradually approach WT steady state levels. Thus, altered regulation of the AhR responsive genes in LA1 may result from the instability of the AhR due to this causative protease redistribution.

**96 EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS AND POLYCHLORINATED BIPHENYLS ON INDUCTION OF CYP1A1, CYP1A2, AND CYP1B1 IN LIVER AND LUNG TISSUES IN ARYLHYDROCARBON RECEPTOR KNOCK-OUT MICE.**
*Osaka Prefectural Institute of Public Health, Osaka, Japan, *Kinki University, Osaka, Japan and *Tohoku University, Sendai, Japan. Sponsored by G. Courterech.

CYP1A1 and CYP1B1 are the most important enzymes in the initial step of oxidation of polycyclic aromatic hydrocarbons (PAHs) to form reactive intermediates and ultimate carcinogens. Arylhydrocarbon receptor (AhR) knock-out mice are shown to be resistant to BENZO(A)PYRENE (B(a)P)-induced carcinogenesis, probably due to the abilities to activate B(a)P by specific P450s in these mice. It is interesting to know which P450s are induced by PAHs and polychlorinated biphenyls (PCBs) in AhR(+/-) and (/-) mice. These mice were injected i.p. with several PAHs and PCBs and were sacrificed at 3 days after the injection. Levels of mRNA of CYP1A1, CYP1A2, and CYP1B1 in liver and lung tissues and activities of liver microsomal P450s were measured. Cyp1a1 mRNA was very low in liver and lung tissues in uncontrol mice and was highly induced in AhR(+/-) mice by PAHs. Cyp1a1, Cyp1a2, and Cyp1b1 in liver and lung tissues and activities of liver microsomal P450s were measured. Cyp1a1 mRNA was increased with 3-methylcholanthrene and Kacechlor 500 in AhR(+/+) mice and was not detected in Cyp1b1. Cyp1b1 was expressed constitutively in liver and lung tissues and was induced by PAHs and PCB in AhR(+) mice. Liver microsomal activities to oxidize several xenobiotics and carcinogens were found to be good indicators for the induction of individual P450s by PAHs and PCBs. Our study sup- port that Cyp1a1, Cyp1a2, and Cyp1b1 are differentially induced in liver and lung tissues of AhR(/+) mice by PAHs and PCBs through AhR and these may be related to the occurrence of liver formation caused by PAHs in the target organs. (Supported in part by Grant from the Ministry of Education, Science, and Culture of Japan, the Ministry of Health and Welfare of Japan, and the Petroleum Energy Center.)
CYTOCHROME P450 2E1 (CYP2E1) IS THE PRIMARY ENZYME RESPONSIBLE FOR ETHYL CARBAMATE (EC) METABOLISM: STUDIES USING CYP2E1-NUI MICE. U. Hoffer1,2 and B. Ghanayem1,2,1. Meharry Medical College, Nashville, TN and 2. NEHS, Research Triangle Park, NC.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>% 14C Derived From EC</th>
<th>% 14C Derived From EC</th>
<th>% 14C Derived From EC</th>
<th>% 14C Derived From EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>KO</td>
<td>80.7 ± 0.2 (%)</td>
<td>91.2 ± 0.3 (%)</td>
<td>78.6 ± 0.2 (%)</td>
<td>65.1 ± 0.3 (%)</td>
</tr>
<tr>
<td>WT</td>
<td>62.3 ± 0.2 (%)</td>
<td>75.5 ± 0.3 (%)</td>
<td>58.9 ± 0.2 (%)</td>
<td>45.2 ± 0.3 (%)</td>
</tr>
</tbody>
</table>

Values are the mean ± SD of 6-9 mice and statistical analysis was performed from corresponding WT and EC only, respectively.

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

S. Uno, T. P. Dalton, A. R. Scott, H. G. Shyng and D. W. Nebert, Center for Environmental Genetics and Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH.

Dioxin (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, TCDD) is a persistent environmental contaminant of unusual potency, owing to the fact that it is negligibly metabolized and it binds with high affinity to the aryl hydrocarbon receptor (AHR). The AHR is a ligand-activated transcription factor responsible for the up-regulation of numerous genes. It is thought that dioxin toxicity might result, at least in part, from chronic activation of one or more of these genes — leading to chronic oxidative stress; as noted among these genes are the cytochrome P450 1 B 1 family members, CYP1A1, CYP1A2 and CYP1A6. Indeed, we have shown that the CYP1A2(-/-) mouse is protected against dioxin-mediated hepatocarcinogenesis. We also found that CYP1A1(-/-) mice are more resistant to BENZOA(APYRNE) (BaP)-induced lethality, compared with CYP1A1(-/-) mice. Although CYP1A1-dependent BaP-induced liver toxicity and metabolism do not correlate with CYP1A1-independent formation of BaP-DNA adducts, we found that CYP1A1(-/-) mice do show reduced levels of BaP-DNA adducts. In the present study, we treated congeneic CYP1A1(+/+) or CYP1A1(-/-) mice with a single dose of dioxin (200 mg/kg, ip) and measured several endpoints of dioxin toxicity. First, all male CYP1A1(-/-) animals died within 4 weeks of treatment, whereas the male CYP1A1(-/-) mice and female animals of both genotypes did not die. The remaining analyses were performed on the surviving female animals. Serum ornithine carbamoyltransferase activities were not significantly different between CYP1A1(-/-) and CYP1A1(+/+) mice. On the other hand, the CYP1A1(-/-) female mice had significantly smaller thymuses and displayed aplastic anemia, compared with that in the CYP1A1(+/+) females. These results suggest that, like the CYP1A2(-/-) mouse, CYP1A1(-/-) mice may show limited protection against dioxin toxicity, but that the degree of protection will likely be tissue-specific.

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

T. Oyama, A. Matsunuma, T. Ise and T. Kawamoto, Departments of Environmental Health, University of Occupational and Environmental Health, Kitakyushu-shi, Japan.

CYP2A6 is a well known enzyme to metabolize nicotine in tobacco smoke. CYP2A6 mRNA is also observed in bronchial epithelial cells. On the other hand, p53 mutation is one of the most common genetic alterations found in human tumors including those of lung. p53 gene mutations lead to a nuclear accumulation of the p53 protein. The incidence of this mutation is related to the smoking index of the patients. The elevated concentration of carcinogens may act to produce these p53 alterations. In this study, we examined 19 patients with non-small cell lung cancer (NSCLC) for CYP2A6 and p53 staining and correlate the findings with lifetime cigarette consumption. We also revealed the relationships between CYP2A6 and p53 immunoactivity in NSCLC. [Materials and Methods] We examined 15 Japanese patients with NSCLC who underwent surgical resection at UOEH. There were 11 men and 4 women ranging in age from 52 to 83 years (mean, 67 years). Ten patients had stage I & II disease, and 9 stage III & IV. Histological typing of the tumors was performed according to the WHO classification (adenocarcinoma: 12 cases, squamous cell carcinoma: 7 cases). The amount of lifetime cigarette consumption was classified by Brinkman Index (BI; cigarette/day * years). Immunohistochemical staining (IHC) was performed on 3 mm paraffin-sections. The monoclonal antibody MAB-2A6 (Genentech) and DO-1 (Oncogene Science Inc.) were used for IHC. We defined as CYP2A1 positive cases when more than 10% of bronchial cells were stained and as positive cases when more than 10% of tumor nuclei were stained. [Results and conclusions] The CYP2A6 positive rate is 50% in NSCLC patients with BI of less than 1000 (66%; 67%) was significantly higher than that with BI less than 1000 (33%; 4/12). The positive rate of p53 expression in NSCLC patients with BI of more than 1000 (86%; 67) was significantly higher than that with BI less than 1000 (25%; 3/12). There was no significant relation between CYP2A6 and p53 expressions. Smoking may induce CYP2A6 and p53 expressions.

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

C. M. Vezina1, S. T. Koury1, J. R. Olson1, and A. I. Dratshuk1, 1Pharmacology and Toxicology, University at Buffalo, Buffalo, NY and 2Biomedical and Clinical Laboratory Sciences, University at Buffalo, Buffalo, NY.

The human cytochrome P450 1B1 (CYP1B1) is a dioxin-responsive CYP capable of metabolizing and activating a variety of procarcinogens into their ultimate carcinogenic forms. Expression of the CYP1B1 gene varies considerably among humans, which may influence individual susceptibility to cancer. In order to determine whether genetic differences in CYP1B1 contribute to variability in gene expression, DNA was isolated from human tissue specimens and the -1139 to -19 region relative to the transcription initiation site was sequenced. CYP1B1 was surveyed for genetic polymorphisms by the methods of Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) and DNA sequencing. Three novel genetic polymorphisms, designated G-787A, T-359C, and C-238T were identified and their generic frequencies were determined by Restriction Fragment Length
Polymorphism (RFLP) analysis. The -139 to +19 region of CYP1B1 was cloned into a reporter plasmid and variants alleles for each polymorphism were introduced by site-directed mutagenesis. Wildtype and variant reporter constructs were analyzed for their ability to induce transcription in vitro in transiently transfected Human MCF-7 breast adenocarcinoma cells. None of the variant alleles demonstrated significant differences in constitutive or dioxin-induced gene expression compared to the wild type constructs. These results indicate that variations in the expression of human CYP1B1 are not influenced by genetic factors in the immediate vicinity of the transcription initiation site. (Supported in part by USEPA R825088 and Multidisciplinary Research Pilot Program, University at Buffalo.)

102 INHIBITION OF TCDD-INDUCIBLE CYP1A1 EXPRESSION IN MURINE HEPATOCYTES BY O, P'-DDT.
J. Y. Kim and H. G. Jeong. Pharmaceutical Research Institute for Proteins, Medical Research Institute, Chonnam National University, Kwanju, South Korea.
Cultured mouse hepatoma Hepa-1c1c7 cells were treated with either o, p'-DDT or TCDD or in combination to assess the role of o, p'-DDT on CYP1A1 expression. o, p'-DDT alone did not affect CYP1A1-specific 7-ethoxyresorufin O-deethylase (EROD) activity. In contrast, the TCDD-inducible EROD activities were markedly induced with co-exposure to TCDD and o, p'-DDT in a dose dependent manner. Treatment with 162780, an estrogen receptor antagonist, did not affect the suppressive effects of o, p'-DDT on TCDD-inducible EROD activity. The TCDD-inducible CYP1A1 mRNA levels were markedly suppressed upon co-exposure with TCDD and o, p'-DDT that is consistent with their effects on EROD activity. A transient transfection assay using dioxin-responsive element (DRE)-linked luciferase and electrophoretic mobility shift assay revealed that o, p'-DDT reduced transcription of the aryl hydrocarbon (Ah) receptor to a form capable of specifically binding to the DRE sequence in the promoter of the CYP1A1 gene. These results suggest that the down regulation of CYP1A1 gene expression by o, p'-DDT in Hepa-1c1c7 cells might be an antagonist of the DRE binding potential of the nuclear Ah receptor but is not mediated through the estradiol receptor. [This work was supported by KFDA Grant (ED2001-1-14) and RCPM.]

103 INACTIVATION OF CYTOCHROME P450 2E1 BY CAPSICAIN.
S. J. Smell, C. A. Reilly and G. S. Yost. Pharmacology & Toxicology, University of Utah, Salt Lake City, UT.
Capsaicin is the principle pungent component of "hot peppers" and of pepper spray products used for self defense. Previous research has demonstrated that dihydrocapsaicin inhibited the cytochrome P450 2E1 (CYP2E1) catalyzes the metabolism of the pain compound 

104 INHIBITION OF CYTOCHROME P450 METABOLISM BY METALLOPORPHYRINS.
Manganese containing metalloporphyrins have been shown to possess a wide variety of antioxidant properties, including the scavenging of superoxide, hydrogen peroxide, lipid peroxides and peroxynitrite. These compounds are currently being developed as therapeutic agents in the treatment of stroke, organ transplant, and cancer. The goal of these studies was to determine whether these compounds interfere with cytochrome P450 metabolism, both in vitro and in vivo. Groups of 4 rats were treated with AEOL-10113 (0.5 mg/kg SC) and 24 hours later benzophenone and resorufin were injected intraperitoneally. AEOL-10113 markedely depressed BRED activity by greater than 90%. Untreated rat liver microsomes were used to determine the in vitro concentration of AEOL-10113 that inhibits BROAD activity by 50% (IC50). AEOL-10113 exhibited an IC50 of 0.2 mM in the BROAD assay. The magnitude containing metalloporphyrin, AEOL-10113, is a potent inhibitor of cytochrome P450. Given AEOL-10113's potent ability to scavenge reactive oxygen species, it is possible that these compounds scavenge the cytochrome P450 activated oxygen intermediate that is necessary for cytochrome P450 catalytic activity.

105 INHIBITORY EFFECTS OF GOLDENSEAL METHYLENEDIOXYMETHYL COMPOUNDS ON HUMAN CYTOCHROME P450s: CYP2C9, CYP2D6, AND CYP3A4.
P. Chatterjee and M. R. Franklin. Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.
A large segment of the population is consuming phytochemicals concomitant with conventional prescription or OTC drugs. The concurrent use of herbal medications carries a potential risk for adverse drug-plant pharmacokinetic interactions, particularly as a result of inhibition of cytochrome P450s. Goldenseal (Hydrastis canadensis), a popular herbal immunomodulatory botanical, contains three methylene-dioxymethylen (MDM) alkaloids, berberine, (-) and (+)-isohydrastine. In human liver microsomes, both berberine and hydrastine, inhibited CYP2D6 but not CYP2C9 and CYP3A4. (IC50 of 1.4 and 1.2, respectively). Berberine aldehyde, isohydrastine, and hydrastine, inhibited CYP3A4 (IC50 of 120 and 120, respectively). The inhibition of CYP3A4 by berberine and hydrastine, is thought to be caused by the formation of a cytotoxic and hydrophobic metabolite.

106 CHARACTERIZATION OF NONSPECIFIC EFFECTS OF PHOSPHOROTHIOATE Oligodeoxynucleotides ON CYTOCHROME P450 3A2 AND CYTOCHROME P450 2C11 IN MALE SPRAGUE-DAWLEY RATS.
G. Pacchecot, S. H. Henry, R. Berryt and A. de Perset. Toxicology & Pharmacokinetics. ISIS Pharmaceuticals, Carlsbad, CA and School of Public Health, San Diego State University, San Diego, CA.
Phosphorothioate oligodeoxynucleotides (PS ODNs) are being studied for treatment of various diseases and are designed to bind specific regions of a target gene to block translation of proteins. PS ODNs can also have nonspecific effects in rodents that lead to immune stimulation as demonstrated by the production of inflammatory cytokines. The purpose of our research is to study the nonspecific effects of PS ODNs on hepatic cytochrome P450s. Cytotoxicity, 2C11 and 3A2 are predominant isoforms of the liver and if altered pose concern for potential drug-drug interactions. Six to eight week old male Sprague-Dawley rats were injected subcutaneously with 5, 15, or 50 mg/kg of ISIS 2105 every other day for a total of three doses. Twenty-four to 30 hours after the last injection, livers, spleens and blood samples were collected. Microsomes were isolated from whole livers and hepatic cytochrome P450 (CYP 3A2 and CYP 2C11) activity was determined by a testostosterone hydroxylase assay. Spleen weights were used as a marker for immune stimulation. Plasma was assayed after a 50 mg/kg dose of PS ODNs measured for cascin 2C11 content of total liver cytokines (IL-2, IL-6, IL-12 and IFN) were not found to affect the activity of specific cytochrome P450 isozymes. Cytosine P450 3A2 and 2C11 are predominant isoforms of the liver and if altered pose concern for potential drug-drug interactions. Six to eight week old male Sprague-Dawley rats were injected subcutaneously with 5, 15, or 50 mg/kg of ISIS 2105 every other day for a total of three doses. Twenty-four to 30 hours after the last injection, livers, spleens and blood samples were collected. Microsomes were isolated from whole livers and hepatic cytochrome P450 (CYP 3A2 and CYP 2C11) activity was determined by a testostosterone hydroxylase assay. Spleen weights were used as a marker for immune stimulation. Plasma was assayed after a 50 mg/kg dose of PS ODNs measured for cascin 2C11 content of total liver cytokines (IL-2, IL-6, IL-12 and IFN) were not found to affect the activity of specific cytochrome P450 isozymes.
CYP 2C11 suggests that cytokines released in response to PS ODN treatment can suppress hepatic metabolism. Additional studies are pending to further study the direct and indirect effects of PS ODN on hepatic metabolism.

107 CYTOCHROME P450 3A2 ANTISENSE MORPHOLINO Oligomer AVI-4472: A 14-DAY TOXICITY STUDY IN MALE RATS.
Cytochrome P450 (CYP) 3A2 is recognized as the rat ortholog of the human CYP3A4 enzyme, which plays a role in the metabolism of more than half of clinically relevant xenobiotics. Phosphorodiamidate Morpholino Oligomers (PMO) are third generation antisense agents that have immunotherapeutic potential. The objectives of this study were to evaluate the toxicity and tissue distribution of CYP3A2 antisense PMO AVI-4472 following daily intraperitoneal injections to male rats for 14 days at two dose levels, and to study the reversibility of observations following a 14-day recovery period. Male Sprague-Dawley rats were injected with vehicle, 3.0 or 30.0 mg/kg/day AVI-4472 intraperitoneally for 14 days. Results indicate that animals in all groups maintained good general health and there were no significant differences in body or organ weights between any groups. Hematological examination revealed an increase in monocyte count to 5222±212 in the high dose group compared with 192±90 in the vehicle group (p<0.05). This index returned to normal following 14 days of recovery. There was a decrease in total bilirubin content to 0.5±0.3 mg/dl in the high dose group compared with 0.6±0.2 mg/dl in the vehicle group (p<0.05). This index did not recover following 14 days of no treatment. Organ distribution of the PMO was identifed in all organs where the target enzyme is detectable, namely kidneys, liver and small intestines (descending order of recovery) at the end of the 14-day treatment period. PMO was also detectable in these organs at the end of the additional 14-day no-treatment period. We conclude that it is feasible to administer AVI-4472 systemically to male rats for term suppression of the CYP3A2 enzyme.

108 ALTERATIONS IN HISTONE ACETYLATION ARE ASSOCIATED WITH PHENOBARBITAL INDUCTION OF THE RAT CYP2B2 GENE.
S. A. Auerbach, F. Liu1 and C. L. Ormerskici.1 (Pharmacology, University of Washington, Seattle, WA and Environmental Health, University of Washington, Seattle, WA).
Phenobarbital (PB) exposure results in the marked induction of a number of biotransformation enzymes in the liver of mammalian organisms, including certain cytochrome P450s (CYP). This process is mediated by the activation of the CAR/RXR nuclear receptor complex and is inhibited by the phosphatase inhibitor olsalazine. Remodeling of chromatin structure is an important event associated with the activation of specific genes by chemical inducers. Previously, it was reported that PB-induced transcripational activation of the rat CYPB2 gene involved tissue-specific alteration of chromatin structure, surrounding a key regulatory element, termed the PB responsive unit (PRU) or PBBS. We used a chromatin immunoprecipitation assay and a primary rat hepatocyte culture system to demonstrate that PB exposure results in enhanced histone H3 acetylation, specifically within the PBBS region of the CYPB2 gene. The changes noted in H3 acetylation were correlated with the binding of RXR to the PBBS. Furthermore, we demonstrate that olsalazine inhibits the changes in histone acetylation and blocks the recruitment of RXR to the PBBS. Characterizing the molecular events associated with xenobiotic induction will enhance our ability to predict the biological and toxicological impact of chemical exposures in affected cells. (Supported by NIH grant GM32281 and the Burroughs Wellcome Fund)

109 EFFECTS OF MATERNAL DIETARY VITAMIN E SUPPLEMENTATION ON DEVELOPMENTAL BIOLOGY AND PHENOTYPIC EMBRYOPATHIES.
J. M. Marcic1, C. S. Oren1 and P. G. Wells1.1 (Faculty of Pharmacy, University of Toronto, ON, Canada and Dept. of Pharmacology, University of Toronto, Toronto, ON, Canada).
The antioxidant vitamin E (VE) given i.p. protect pregnant mice from embryonic DNA oxidation and teratogenesis caused by the reactive oxygen species ROS-initiating drug phenytoin (PHT). Also, maternal dietary supplementation with low (0.1-2.0%) but not high (10%) levels of VE reduces both endogenous and PHT-enhanced DNA oxidation. Here, we examined the effect of maternal dietary VE (all-α-tocopherol-acetate) supplementation on normal embryonic development and PHT teratogenesis. Virgin CD-1 females were placed on either a normal or VE-supplemented (0.1%, 2.0%, or 10% [w/w]) diet for 4 weeks and mated. Dams were administered PHT (65 mg/kg i.p.) or its saline vehicle on gestational days (GD) 12 and 13 at 0900 hr. In vehicle controls on GD 19, spontaneous fetal resorption incidence was reduced 84% by 0.1% dietary VE, by 82% by 2% VE (p<0.05), and by less than 10% by 10% VE. In PHT-treated animals, 0.1% VE reduced PHT-enhanced fetal resorptions by 68% (p=0.02), but this protective effect was lost at higher VE doses. PHT-enhanced fetal weight loss (p=0.01) was reduced to the level of vehicle controls at 2.0% and 10% VE. VE did not significantly alter PHT-initiated fetal cleft palate incidence, which was 50% in vehicle controls (p=0.02), and higher VE diets of 2.0% and 10% non-significantly enhanced postpartum lethality in a dose-dependent fashion. The VE effects on resorptions are congruent with those on DNA oxidation, where 0.1% VE, but not higher doses, provided optimal protection from both endogenous and PHT-enhanced DNA oxidation. The protection by VE suggests that ROS involvement in at least some PHT embryopathies, specifically endochondral bone development, and that the biphasic nature of VE protection suggests caution in the design of therapeutic strategies. Results from these vehicle controls show that even endogenous levels of ROS are embryopathic, suggesting developmental benefits from maternal VE supplementation (Support: CHIR)

110 DEVELOPMENTAL TOXICITY OF DI- AND TETRACHLOROETHANE AND DICHLOROPROPANE IN THE RAT WHOLE EMBRYO CULTURE SYSTEM.
Disacquation of drinking water with chlorine results in numerous chlorinated byproducts (DBPs). Among these are the di-substituted phenol and the di- and tetra- substituted ethanes. We chose to evaluate the developmental toxicity of 1,3-dichloropropane (1,3-DPP), 2,2-dichloropropane (2,2-BPP), 1,3-dichloroethylene (1,3-DCE) and 1,2,2-trichloroethylene (TCE) in rat whole embryo culture (WEC) due to their presence in drinking water and a lack of developmental toxicity data available for these compounds. Gestational day (GD) 9 rat embryos were exposed to 2.5-7.6 mM 1,3-DP; 8.7-19.4 mM 2,2-BPP; 8.9-23.8 mM 1,3-DCE; and 0.5-2.9 mM TCE for 48 hours and then evaluated. All compounds were dysmorphic in rat WEC. 1,3-DP was dysmorphic at all concentrations ≥3.8 mM and embryolethal at concentrations ≥1.1 mM. Anomalies were opened neural tube, rotational and eye defects. DEVSC, HL, SN and CRL were significantly reduced at concentrations ≥3.8 mM. 2,2-BPP was dysmorphic in rat WEC at concentrations ≥1.6 mM and embryolethal at concentrations ≥14.5 mM. Anomalies were predominantly open neural tube, heart defects and rotational defects. DEVSC was significantly reduced at concentrations ≥1.05 mM. 1,3-DCE was significantly dysmorphic at 17.9 mM but also significantly embryolethal (35%). The anomalies were eye defects but there was overall delayed embryonic development at the higher exposure concentrations. TCE was dysmorphic at concentrations ≥1.4 mM and embryolethal at concentrations ≥2.4 mM. Anomalies were primarily heart eye and rotational defects. The relative developmental toxicity for the four compounds was TCE>1,3-DP>2,2-BPP>1,2. TCE, 1,2. The pattern of anomalies was inconsistent between the 4 compounds suggesting that their toxicity is probably expressed through different mechanisms. The concentrations of these DBPs required to induce dysmorphogenesis are much higher than those for the halocarbons, another class of DBPs that we have studied extensively. This abstract does not reflect EPA Policy.

111 A NEW ANIMAL MODEL FOR AUTISM: EFFECTS OF EARLY EXPOSURE TO SODIUM VALPROATE IN MICE.
A. K. Halladay1, F. MaRae1, K. R. Reish1 and G. C. Wagner. Pharmacol and Toxicol, Rutgers Univ, Piscataway, NJ and by the Mayo Clinic, Piscataway, NJ.
Early exposure to environmental agents may have a role in the etiology of pervasive developmental disorders (PDD) including autism. Such exposure may cause neurobehavioral retardation or regression (wherein an acquired behavior is lost), or irritations (wherein acquired behaviors are overwhelmed by new behaviors) (2). Teratogenic effects of the antiepileptic drug sodium valproate (VPA) have been well documented in the form of neurological deficits and decreases in Purkinje cell number, which have been associated with autism. In the present study, PND 14 male BALB/c mice were tested for skills corresponding to cerebellar development. Following initial testing, mice were treated with 200 mg/kg i.p. sodium valproate (VPA), followed on these tasks until the second day. 2, 3, 4 and 5 days later and examined for emergence of habituation of motor activity and spatial learning ability on 23-26. Treatment with VPA on day 14 produced a regression in mid-air righting as well as an established negative geotropism. This is, while pups had developed these abilities before treatment, these behaviors were lost for up to 72 hours after VPA administration. In addition, treated pups showed a delay in the navigation of a water maze task with no alterations in the habituation of motor activity. VPA-induced changes in developmental behaviors were not observed when...
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EFFECTS OF LOW LEVEL TECHNICAL CHLORIDE ON SELECTED SEX STEROID MEDIATED PARAMETERS IN PREPUBERTAL RATS.
F. O. Johnson, V. E. St. Omer, K. M. Minnig, B. C. Dutri, M. M. Mansour, R. D. Mosha and E. O. Abbadi., Biological Sciences, Tuskegee University, Tuskegee, AL; Pharmacology, University of Kansas City, MO; and Veterinary Physiology, Seikou University of Agriculture, Seikou, Tanzania.
Sponsor: R. Dalvi.

Technical chloride is a highly persistent toxic environmental pollutant and a formerly used pesticide. Humans and other mammals are being exposed to low levels of chloride and their long-term effects during critical developmental periods are not well documented. This study evaluated the effects of technical chloride on sex steroid mediated parameters (anogenital distance [AGD], testicular descent, preputial separation, testes, seminal vesicles, prostate, testosteron, uterus and ovaries) of prepubertal rats in a multi-generational study. Sprague-Dawley rats. F1 adult and their F2 offspring were exposed, pre- and postnatally, to daily levels of chloride (0.0, 0.125, 0.25 and 1.0 mg/kg) until termination. F2C rats on PND 1 manifested increases (p<0.05) in AGD, a biomarker of androgenicity. Treatment also delayed the onset of balano-preputial separation and testicular descent (anogenital biomarker) in F2C males. While in females, chloride delayed vaginal opening (an estrogen dependent parameter). Plasma testosterone concentrations on PND 30 were reduced (p<0.05) in treated males but not females. The physical maturation parameters (balano-preputial separation, testicular descent and vaginal opening) correlated with changes in plasma testosterone concentrations. Dose-related reductions occurred in absolute and relative organ (brain weight ratio of testes, uterus and liver). In conclusion, chloride exhibited androgenic-like activities in utero, and both anti-androgenic (male) and anti-estrogenic (female) responses during prepupetal development. Therefore, humans may be at risk from chloride as an endocrine disruptor. (Supported by: MHPF/ATSDF U50/AT/532266008).

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EMBRYOTOXICITY ASSOCIATED WITH SPERMATOZOA BENZO(A)PYRENE-DNA ADDUCTS TRANSFERRED TO FERTILIZED EGGS.
S. B. Hogeve and P. Collodi., Veterinary Pathobiology & Animal Science, Purdue University, West Lafayette, IN.

Sperm benzo(a)pyrene-DNA adducts can be transferred to fertilized embryos. Using 3HJy-postlabelling, we have previously identified a DNA adduct ([+) anti-BPDE-damaged DNA in testis sperm treated with herotox[pyrene dield epidioxide (BPDE)]. When BPDE-treated sperm were incubated with normal mouse eggs, the same adduct was found at 24h. To assess developmental effects, trout sperm were incubated with 50 ng/mL BPDE or saline with 1% ethanol and used to fertilize untreated trout eggs. For 10d, and at 15d post-fertilization, eggs were examined microscopically. Eggs hatched at 20d and fry were weighed. At 24h post-fertilization, eggs in both groups had visible cell masses indicating 100% fertilization. By 72h, gastrulation was observed in 96% of PBS embryos but was delayed in embryos from BPDE-treated sperm with gastrulation in only 77%. At 5d, approx. 95% of PBS embryos had formed a complete body axis compared to approx. 60% of BPDE embryos. Between days 6 and 10, approx. 85% to 95% of PBS embryos (n=50 each day) had normal development with 98% having eye spots & beating hearts on day 10. In contrast, only approx. 50% of BPDE embryos (n=50 each day) appeared normal. Of the abnormal, half had no noticable development while the others had truncated body axes with disorganized cell masses. At 15d, approx. (of 212) PBS embryos had normal development compared to 32% (of 143) from BPDE-treated sperm. Eggs from PBS-treated sperm hatched at 72% (1309 of 1912 eggs) with normal trout fry, while only 8% (85 of 1137 eggs) from BPDE-treated sperm hatched. Those that hatched appeared & weighed the same as PBS fry. Non-viable eggs from BPDE-treated sperm had varying forms of abnormal development ranging from none, to elongated masses of cells, to various degrees of truncated development. Sperm adducts did not affect fertilization rate, but were associated with embryotoxicity: delayed gastrulation; incomplete body axis formation; abnormal development; and decreased hatch. Assistance was provided by the Bodine State Trout Hatchery, IN Department of Natural Resources.

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CONTRASTING DEVELOPMENTAL ROLES FOR ATAXIA TELANGECTASIA-MUTATED (ATM) IN MODULATING THE EMBRYONIC RESPONSE TO ENDONUCLEASE AND XENOBIOTIC-ENHANCED REACTIVE OXYGEN SPECIES (ROS) IN ATM KNOCKOUT MICE.
Y. Bhullar and P. G. Wills., Faculty of Pharmacy, Univ. of Toronto, Toronto, ON, Canada and *Dept of Pharmacology, Univ. of Toronto, Toronto, ON, Canada.

The ATM protein is a key ROS sensor and signal transducer in the genotoxic stress response pathway, and can trigger p53-dependent DNA repair. We have shown that pregnant Atm knockout mice are more sensitive to the embryotoxic effects of ionizing radiation, and here we determined if they are similarly more susceptible to the embryotoxic effects of the ROS-initiating drug phenytoin. Female wild-type (+/+) or heterozygous (+/-) Atm knockout mice were mated with +/- males, and dams were treated with phenytoin (65 mg/kg ip) or its saline vehicle on gestational days (GD) 1 and 12 (p-gal-GD1). Resorptions and fetuses were genotyped and characterized on GD19. With untreated (vehicle controls) +/- dams, fetal body weight was lower in homozygous Atm null (-/-) fetuses compared to +/- and +/+ littermates (p<0.05). Independent of treatment, there were opposite Atm gene dose-dependencies in pre- and post-implantation death. Implantations were 45% lower in +/- than +/+ (p<0.05). In contrast, in +/- dams, no resorptions had a +/- genotype compared to a substantial incidence with +/- (45%) or +/+ (55%) genotypes in +/+ and +/- dams (p<0.05). This strain was relatively resistant to phenytoin embryopathy. In +/- dams, phenytoin did not cause fetal body weight loss or enhance resorptions or postpartum lethality. However, with +/-, fetal body weight loss was enhanced by phenytoin in -/- Atm fetuses compared to both their +/+ and +/- phenytoin-exposed littermates (p<0.05), and to +/- vehicle controls (p<0.05). Conversely, phenytoin-initiated postpartum lethality was increased in offspring compared to +/- dams (p<0.05). These results suggest that ATM plays important and contrasting developmental roles, and provide the first evidence that ATM affects protection against some, while enhancing other, embryotoxic effects of ROS-initiating teratogens. (Support: Canadian Institutes of Health Research)

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

Maternal exposure to high doses of TCE has been implicated in eye malformations in fetal rats, primarily micro-/anophthalmia. Coincident with a cardiac teratogenicity of TCE and its metabolites, TCA and DCA, the potential for these compounds to induce ocular terata was examined. Pregnant Sprague-Dawley rats were orally treated on gestation days 6-15 with bolus doses of either TCE (500 mg/kg), TCA (300 mg/kg), DCA (300 mg/kg) or the prototereogen, all-trans retinoic acid (RA, 15 mg/kg). The doses of gestation day 21 fetuses were only examined grossly for external malformations, but nearly 100% fetal heads were sectioned using a modified Wilson's technique and subjected to computerized morphometry that allowed for the quantification of lens area, globe area and interocular distance. Cross species malformations were essentially absent in all treatment groups except for the RA group in which 29% of fetuses exhibited micro-/anophthalmia. Lens area, globe area and interocular distance were significantly reduced in the TCA, DCA and RA treatment groups, but not the TCE treatment group. However, the reduced body weights of TCA-, DCA- and RA-treated fetuses appear sufficient to explain the changes in these dependent variables based upon these preliminary results. Neither TCE nor its acid metabolites can be characterized as ocular teratogens. With respect to TCE, this differs from earlier reports generated with a different rat strain and larger doses.

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

Uterine blood flow (UBF) is the major determinant of oxygen and other essential nutrients required prior to the fetus. Alterations of UBF during pregnancy may impair fetal development. In recent years, many techniques have been developed for organ blood flow measurement, including ultrasound flowmetry. The aim of the present
study was to evaluate this technique for quantifying UBF in pregnant rats. Maternal UBF and heart rate were measured on the 1st day of pregnancy and daily thereafter until parturition. UBF was measured using a 1 mm flow probe (125, Transonic Systems, Ithaca, NY). Absolute and relative UBF were 2.96 ± 0.16 ml/min and 0.029 ± 0.015 ml/min/g of gravid uterus, respectively, and heart rate was 393 ± 36 bpm. These values were within the expected range reported in the literature. We then investigated the effects of UBF on phenylephrine, a postganglionic alpha-adrenergic agonist agent which induces uterine vasoconstriction. Pregnant Sprague-Dawley rats on GD21 were given subcutaneous 12.5 mg/kg phenylephrine and UBF was measured continuously for up to 25 minutes after injection. We found that UBF decreased up to 100% when compared to the control values recorded before injection, whereas HR was unchanged. Overall results indicated that the ultrasonic transit time flowmeter system appears to be a promising technique to determine UBF in pregnant rats and to identify negative effects induced by vasoconstrictive agents.

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


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

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

R. C. Grasty1, B. E. Grey1, J. Thibodeaux1, C. Lau2 and L. M. Rogers1.

1 Reproductive Toxicology Division, NHEERL, ORI, EPA. Research Triangle Park, NC and 2 Department of Toxicology, UNC Chapel Hill, Chapel Hill, NC.

PFOS belongs to a class of fluorinated organic chemicals with broad industrial use. Despite a reduction in the manufacturing of products containing PFOS and many of its metabolic precursors, levels of accumulated PFOS in the environment warrant evaluation of its potential for toxicity. The present study investigated the critical period for increased neonatal mortality caused by PFOS. Timed-pregnant Sprague-Dawley rats were treated by oral gavage with 25 mg/kg PFOS (potassium salt dissolved in 0.5% Tween-20) for four consecutive days during either GD 2-5, 6-9, 10-13, 14-17 or 17-20. Controls were given vehicle daily throughout GD 2-20. Blood samples were taken the day after the last day of dosing to determine maternal PFOS levels. Dams were allowed to continue nursing their offspring until postnatal day 21. Maternal weight gain was reduced in treated animals during dosing and returned to near control levels by term. Similar patterns were seen in food and water consumption. Average litter size was normal in all groups compared to controls with no significant difference in average pup weight on the day of birth. PFOS induced decreases in neonatal survival were seen for each group while controls remained at about 100% survival. Mortality increased as the dosing period fell later during gestation with the latest group (GD 17-20) approaching 100% mortality. All deaths occurred before PD 4 with the most taking place during the first 24 hours. Growth retardation was observed in surviving pups of treated dams and gross dissection suggested underdeveloped lungs. A second study compared perinatal survival between dams treated with 100 mg/kg PFOS on GD 17 or 20. Both groups had a high rate of neonatal death with no significant difference between the two. Analysis of maternal PFOS levels are in underway. These results demonstrate that the critical period for PFOS induced neonatal mortality in the rat occurs near birth. This abstract does not reflect EPA policy.

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

C. Lau1, J. M. Rogers1, J. R. Thibodeaux1, R. G. Hanson1, B. E. Grey1, B. D. Babee2, J. Richards3 and J. L. Butechhoff3. 1 Reproductive Toxicology Division, USEPA. Research Triangle Park, NC; 2 Exp. Toxicology Div., USEPA. Research Triangle Park, NC; and 3 Med. Dept., 3M, St. Paul, MN.

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

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

J. Butechhoff2, R. York3, A. Sessa2 and D. Luchter2. 13M Medical Development, Corporate Toxicology, 3M, St. Paul, MN and 2 Argus Research Division, Charles River Laboratories, Horsham, PA.

To better define the dose-response of perinatal mortality observed in prior reproduction studies with perfluorooctanesulfonate (PFOS), female rats were administered perfluorooctanesulfonate oral intubation at dose levels of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg/d for 42 days prior to mating to untreated males, during mating and through the end of gestation (ceasean section) or four days of lactation (natural delivery). At day 21 of gestation, fetuses from eight litters in the 0, 1.6, and 2.0 mg/kg/d dose groups were taken by cesarian section. All remaining dams were allowed to litter, and were sacrificed with litter on day 5 of lactation. For all or some of the groups, PFOS sera levels, serum lipids, glucose and thyroid hormones were measured at both time points. Milk cholesterol and liver PFOS were also monitored on lactation day 5. Significant decreases in cholesterol and increases in LDL were observed in all fetuses from the 1.6 and 2.0 mg/kg/d PFOS dose groups on day 21 of gestation. On day 5 of lactation, dams had significantly decreased serum cholesterol at all dose levels, significantly decreased serum triglycerides in the 1.6 and 2.0 mg/kg/d dose groups, and significantly increased serum glucose in the 2.0 mg/kg/d dose group. Although T4 (free and total) appeared to be decreased when measured by RIA, free T4 was normal across all dose groups (dams) when measured using equilibrium dialysis. T3 was low at 1.2 mg/kg/d and above in dams but not in fetuses and pups. TSH was not elevated and thyroid hormone dose groups showed an increase in relative liver weight, a decrease in body weight gain, a decrease in food consumption, and a slight decrease in the duration of gestation. Pup growth was decreased at 1.2 mg/kg/d and higher. Viability indices for pups from the 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg/d groups were 97, 98, 95, 89, and 82, 42, and 17 percent, respectively.
121 PERFLUOROOCTANESULFONATE-INDUCED PERINATAL MORTALITY IN RAT PUPS IS NOT A RESULT OF REDUCED SERUM LIPIDS.
D. Luebker, R. York, A. Seo et al, and J. Weisbroth. 3M Medical Department, Corporate Toxicology Center, St. Paul, MN and lntergen Research Division, Charles River Laboratories, Horsham, PA.
This study was designed to test the hypothesis that hypolipidemia causes perfluorooctanesulfonate (PFOS)-induced perinatal mortality in rats. Female rats were supplemented with either 500 mg/kg of mevalonic acid lactone (MAL) twice daily or 500 mg/kg cholesterol (CHOL) once daily and compared to non-supplemented controls. Dose groups consisted of 0.5% vehicle control, CHOLvehicle control, MALvehicle control, 1.6 mg/kg/d PFOS, 1.6 mg/kg/d MAL plus 1.6 mg/kg/d CHOL, 2.0 mg/kg/d PFOS, 2.0 mg/kg/d MAL plus 2.0 mg/kg/d CHOL, and 2.0 mg/kg/d PFOS plus 2.0 mg/kg/d MAL. Female rats were dosed for 42 days prior to mating to untreated males, during mating and through the end of gestation or four days of lactation. Fetuses from eight litters per dose group were taken by cesarean section on day 21 of gestation. All remaining dams were allowed to lactate, and were sacrificed with litter with litter on day 5 of lactation. For all or some of the groups, PFOS xeral levels and serum lipids, glucose, and mevalonic acid were measured at both time points. For fetuses and gestation day 21 dams, all clinical chemistry parameters remained at or above control levels with the exception of triglycerides (TRIG), which were decreased in fetuses in the MAL-supplemented 2 mg/kg dose group. On lactation day 5, dams had decreased CHOL in all PFOS-treated groups and decreased TRIG in the PFOS-only and CHOL-supplemented PFOS groups. Pups in the CHOL-supplemented 2 mg/kg/d group had decreased TRIG. MAL or CHOL supplementation was unable to prevent or mitigate the perinatal mortality. Viability indices for the 0.5% vehicle control, CHOLvehicle control, MALvehicle control, 1.6 mg/kg/d PFOS, 1.6 mg/kg/d MAL plus 1.6 mg/kg/d CHOL, 2.0 mg/kg/d PFOS, 2.0 mg/kg/d MAL plus 2.0 mg/kg/d CHOL were 97, 99, 98, 49, 41, 42, 17, 1, and 14 percent, respectively. Results suggest that the observed perinatal mortality is not a result of hypolipidemia in the perinatal period.

122 CADMIUM ABSORPTION AND ITS RELATIONSHIP TO DIVALENT METAL TRANSPORTER-1 IN THE PREGNANT RAT.
T. K. Leazer, Y. Liu and C. D. Klaassen, Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.
In pregnancy, the maternal gastrointestinal tract undergoes many physiological and biochemical changes that accommodate the increased demand for essential nutrient absorption and transfer to the fetus. Increased absorption of nonessential metals such as cadmium (Cd) has been reported in pregnant mice in comparison to nonpregnant mice, although the mechanism is not understood. Divalent metal transporter-1 (DMT-1) is localized on the apical membrane of the intestinal epithelial cell and has broad substrate specificity for many divalent metals including Cd. The purpose of this work was to determine whether there is increased Cd absorption in pregnant rats compared to non-pregnant rats, and whether this correlates with up-regulation of DMT-1 expression. Timed pregnant and non-pregnant female Sprague-Dawley rats were administered 0.4 μmol CdCl2 in 4 ml saline/kg (ICGCl) by oral gavage on gestation day (gd) 19. Tissues were collected on gd 20, weighed and assessed for Cd content (values expressed as pmol Cd/g tissue). There was greater accumulation of Cd in duodenum than in jejunum and ileum in both pregnant and non-pregnant rats. However, the amount of Cd in all three portions of the small intestine was higher in pregnant than non-pregnant rats (duodenum 0.74 vs. 0.415 g/m; jejunum 2.12 vs. 0.96 g/m; ileum 24.4 vs. 10.7 g/m). In addition, more Cd accumulated in the liver and kidney of pregnant than non-pregnant rats (567 vs. 249 and 81 vs. 40, respectively). The time course of duodenal DMT-1 mRNA expression was determined in pregnant rats by the branched DNA signal amplification method. DMT-1 mRNA levels were about 6-fold higher in duodenum of pregnant than non-pregnant rats. Duodenal DMT-1 mRNA levels in pregnant rats increased from gd 15 through gd 21, as compared to the non-pregnant rats. The correlation between Cd absorption and observed pregnancy suggests the importance of DMT-1 in the absorption of Cd in pregnant females. (Supported by NIH ES-01142 and ES-07079)

123 REGION SELECTIVE CHANGES OF N-CADHERIN EXPRESSION FOLLOWING IN OVA AND IN VITRO METHYLMERCURY EXPOSURE TO DEVELOPING CHICK BRAIN.
P. M. Day and C. P. Gifredo, 1Neurotoxicology and Experimental Neuropathology Laboratories, Department of Pharmacological Sciences, Wilkes University, Wilkes-Barre, PA and Jefferson Medical College, Philadelphia, PA.
Methylmercury (MeHg) exposure during embryogenesis disturbs brain development and results in decreased neurological potential. The mechanism(s) underlying these effects remain unclear. Accumulating evidence suggests that perturbation in the expression of neural adhesion molecules, several families of cell surface proteins which facilitate cellular migration and cytodifferentiation, contribute to brain dysmorphismogenesis. Prominent in brain development is N-cadherin, an adhesion molecule whose developmentally-regulated expression is required for proper formation and topographic patterning of the central nervous system. To determine whether changes in N-cadherin expression occur in chick brain following developmental MeHg exposure, embryonic Day 11 chicken eggs were injected with concentrations of MeHg ranging from 0.2 to 2 μM and killed 48 hours later. In parallel studies, cortical and cerebellar cultures were prepared from embryonic D11 chicks, exposed to 0.2 to 2 μM MeHg for 48 hours in vitro. Protein and mRNA were extracted from cerebellar and cortical samples and analyzed for N-cadherin expression using Western blotting and RT-PCR, respectively. Semi-quantitative PCR analysis revealed significant reductions in N-cadherin in both in vivo and in vitro exposed chick cerebellum mRNA 48 hours post-treatment, as compared to controls. In contrast, cortical mRNA was not significantly altered in in vivo or in vitro. Western blotting for N-cadherin protein in brain tissue regions was in agreement with mRNA data. The results indicate that MeHg can alter N-cadherin levels in a brain region-selective manner. Disruption of cadherin-dependent morphogenetic events may contribute to the neuroanatomical and neurological defects observed following developmental metal neurotoxicity. Supported by Wilkes University Type I and II research grants.

124 EFFECTS OF RETINOIC ACID ON CELL DEATH DURING IN VITRO CHORDONGENESIS.
G. E. Tillman, K. A. Ageyukum, G. L. Anderson and M. A. Smith, Environmental Health Science, University of Georgia, Athens, GA.
Programmed cell death occurs normally in developing limbs helping to give shape and form to the digits and limb. Retinoic acid has been shown through histochemical staining and whole animal studies to affect chordogenesis and cell death. This study examines the effects of retinoic acid on in vitro chordogenesis and whether this effect is mediated through death of chordochordomal cells from stage 25 embryonic chick limb buds grown in micromass culture for 5 days. To determine overall effect on chordogenesis, cultures were continuously exposed to retinoic acid concentrations ranging from 10-3 M to 10-7 M. Propidium iodide, an indicator of chordogenesis, was determined by fixing the cultures, staining the cells with alician green and visualizing spectrophotometrically at 600 nm. To examine cell death, mesodermal cells from forelimb and posterior limb bud were grown in micromass tissue cultures and exposed to 10-7 M retinoic acid for 48 h beginning on day 5 and day 7 of culture. At the end of the culture period, supernatants were removed from the cultures and lactate dehydrogenase (LDH), an indicator of cell death, was measured. To determine whether cell death was a result of apoptosis, cell cultures were lyzed and DNA was observed for 15% agarose gel for apoptotic DNA fragmentation. Retinoic acid treatment for the five-day period decreased propidium iodide content as shown by alician green staining (49.8%, 39.3%, 28.6%, and 15.6% of vehicle control). Levels of cell death as assessed by LDH were increased in treated cultures compared to control cultures (119.3% of control at day 7 treatment). Gel electrophoresis shows that apoptotic DNA fragmentation occurs at earlier times (7 and 9 day set 14 day) which are the conditions used. These results suggest that exogenous retinoic acid affects in vitro chordogenesis by increasing inappropriate apoptotic cell death of chordocytomes. (Supported in part by Hatch Funds to MAS and the Interdisciplinary Toxicology Program, UGA).

125 INHIBITION OF ANGIogenesis BY 2, 5, 7, 8- TETRACLORIDIBENZOP-4-DIOXIN (TCDD) IN CULTURED EMBRYONIC HEARTS.
L. D. Kiritsis and M. K. Walker, College of Pharmacy, University of New Mexico, Albuquerque, NM.
It has been documented that TCDD reduces coronary angiogenesis on incubation day 0 in chick embryo hearts in vivo. In this study we investigated effects of TCDD on coronary vasculogenesis and angiogenesis at an earlier time point of development. Ventrices from 6-day-old control and TCDD-treated (0.3 pmol TCDD/g) embryonic chick hearts were cultured on a three-dimensional collagen gel. After 2 days of growth in normal medium and 3 days of starvation in low serum medium (0.5% fetal bovine serum), the heart explants were fixed, washed and blocked. Endothelial cells, which migrated out from the heart explants and formed tubes, were identified by wheat germ agglutinin (WGA) antibody using immunohistochemistry. Three-dimensional images of tubes growing into the collagen gel were obtained using confocal microscopy. Tube formation was also observed by electron microscopy (EM). Total length of the tubes was measured as a function of the explant diameter with use of Image Tool software. Confocal and EM images showed that the cells growing outward from the myocardial explants formed tubes in culture. Immunohistochemistry revealed that the cells which formed tubes in culture were endothelial in origin, based on the positive WGA staining. Vascular growth, as measured by total tube length divided by explant perimeter, was signific-
cantly reduced in explants from TCDD-treated chick embryos as compared to explants derived from control embryos. These data suggest that TCDD may inhibit coronary angiogenesis in vivo by affecting vascular precursor cells present earlier in cardiac development. Future studies will use this explant culture system to elucidate the function of hypoxia in coronary angiogenesis and the relationship between hypoxia and TCDD in this process. Supported by NIH grant ES-09894.

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

C. T. U. Baranad, E. I. Lassman and B. T. Zeoller, Department of Biology and Molecular and Cellular Biology Program, University of Massachusetts, Amherst, MA.

Polychlorinated biphenyls (PCBs) are known to be neurotoxic in experimental animals and are associated with neurological deficits in humans. In addition, PCBs are known to reduce circulating levels of thyroid hormones in experimental animals and in humans are associated with measures of thyroid hormones. These observations have led to the hypothesis that PCBs exert at least some neurotoxic effects by reducing circulating levels of thyroid hormones during neural development. Because our Lab has found that thyroid hormone of maternal origin can affect Notch signaling in the fetal rat brain, we tested the hypothesis that maternal exposure to PCBs can affect Notch signaling in the fetal rat brain. We used HES-1 and HES-5 mRNA expression as markers of Notch activity. Pregnant rats were given Aracor 1254 (AECROL 1254) at 8.25 weeks gestation. HES-1 and HES-5 expression were observed at gestational day 2 (G2) and prepared for measurement on gestational day 3 (G3) using in situ hybridization. We found that AECROL 1254 produced a dose-dependent increase in HES-1 expression in the forebrain region of the fetal cortex, but did not significantly change HES-5 expression. Moreover, these effects were observed in the same region of the cortex that was found to be affected by manipulating maternal thyroid status. Since HES-1 expression is associated with differentiation of radial glia from neurotransmitter precursor cells in the ventricular zone of the fetal cortex, these results suggest that PCB exposure may result in a shift toward the production of more glial and fewer neurons in the early cortex. Studies are under way to test this hypothesis and to determine whether effects persist into adulthood. Supported by ES01026 and EPA STAR Graduate Fellowship.

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

R. B. Mahannah, N. S. Bridge, F. S. Kodavanti and N. H. Zawia, Biomedical Sciences, University of Rhode Island, Kingstons, RI; Biology, Savannah State University, Savannah, GA and Neuroendocrinology Division, USEPA, Research Triangle Park, NC.

Polychlorinated biphenyls (PCBs) are a class of persistent chemical pollutants prevalent in the environment despite the ban of their use for decades. Disruptions in brain development and cognition are among the neurotoxic manifestations of PCBs. The cellular and molecular basis for PCB-induced developmental neurotoxicity is still unclear, however, a series of in vitro and in vivo studies have revealed that disruption of Ca2+ homeostasis and Ca2+ -mediated signal transduction plays a significant role. The culminating event in a variety of signal transduction pathways is the regulation of gene expression. Therefore, we examined the DNA-binding of several transcription factors in order to identify those that are involved in signal transduction and transcriptional coupling in the developing brains of PCB-exposed animals. Pregnant rats (Long Evans) were exposed perinatally to 0 or 6 mg/kg/day of Aracor 1254 (AECROL 1254) (Accustandard Inc., Lot # 234-191) from gestation day 6 through postnatal day (PND) 21. On specific PNDs (viz., 7, 14, 21, and 60, the cerebellum was isolated. The DNA-binding of various transcription factors: Sp1, AP1, NF-KB and CREB was monitored using gel mobility shift assays. While an overall transient increase in the DNA-binding of all transcription factors was observed following birth, this period was characterized by a pronounced induction in Sp1 and NF-KB DNA-binding. The distinct changes in Sp1 and NF-KB DNA-binding suggest that they may be involved in mediating PCB-induced perturbations in gene expression that occur during perinatal brain development. (The abstract does not necessarily reflect USEPA policy).

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

E. A. Lassman and B. T. Zeoller, Molecular and Cellular Biology, University of Massachusetts, Amherst, MA.

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

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

K. A. Miller, 'T. P. Dalton, C. V. Vareches and D. W. Nebert, Environmental Health, University of Cincinnati, Cincinnati, OH and Developmental Biology, Children's Medical Center, Cincinnati, OH.

Toxicity of polychlorinated biphenyls (PCBs) first became evident following the 1973 Michigan disaster in which about 1,000 lbs of Firemaster was inadvertently added to cattle feed. Cows and their calves became sick, and thousands of farm animals were destroyed. Humans eating dairy products also manifested neurological symptoms, including a possible association with lowered I.Q. in children who were in utero at the time. Neonatal lethality from maternal PCB exposure has also been reported in the offspring of mink fed diets containing Firemaster. We have chosen to assess in utero toxicity in pregnant C57BL/6j (B6) mice (having the high-affinity aryl hydrocarbon receptor (AHR) and B6.D2-Abhd congenic mice (having the poor-affinity AHR on a B6 background), treated at gestational day (GD) 15 with 100 mg/kg coplanar 5, 4, 3, 4', 5 -hexabromobiphenyl (CBB) or non-coplanar 2, 4, 6, 2', 4', 5'-hexabromobiphenyl (CBBH). In controls, nCBB-treated B6 and B6.D2-Abhd mice and CBBH-treated B6.D2-Ard mice, 100% of offspring survived birth and the weaning period. In CBB-controlled B6 mice, however, 100% of the pups died within the first 5-7 days post partum. Histologic exam of the B6 pups showed lesions in the liver with white-cell infiltrates, indicative of hepatic failure, whereas the mother's liver appeared normal. Northern blot and RT-PCR analysis confirmed CYP1A1 mRNA induction in fetal liver and brain at GD18 in CBBH-treated B6 but no other groups. The mechanism of neonatal toxicity remains unknown, but may appear to be AHR-mediated. Lower doses of nCBH and behavioral tests in surviving pups at 60 days of age are planned. Supported, in part, by NIH grants PO5 ES00696 and ROI ES08147. review.
p<0.05), but did not protect against decreases in yolk sac diameter and crown rump length. The protective effects were not observed using sense or nonsense controls, or antimmune vehicle, suggesting that NF-kB-mediated signal transduction may play a role in the mechanism of phenytoin embryopathy. (Support: Canadian Institutes of Health Research)

131 P53 MODULATION OF PHENYTOIN EMBRYOPATHIES IN P53 KNOCKOUT MICE.
A.W. Wong1 and P.G. Wall1,2. 1Faculty of Pharmacy, Univ. of Toronto, Toronto, ON, Canada and 2Dept. of Pharmacology, Univ. of Toronto, Toronto, ON, Canada.

The tumour suppressor protein p53 facilitates DNA repair and protects against the embryopathic effects of DNA-damaging drugs like BENZO(A)PYRENE, which enhances embryonic reactive oxygen species (ROS) formation. We previously found that p53 protected against fetal resorptions and postpartum lethality (PPL), caused by the ROS-initiating anticancer drug phenytoin given on gestational days (GD) 12 and 13 (plus GD 14). Since p53 expression decays during embryogenesis, we determined here whether its modulation of phenytoin embryopathy differed when phenytoin was given earlier in gestation. Virgin female heterozygous (+/-) p53 knockout mice were mated with p53/-; males, dams were administered phenytoin (65 mg/kg ip) or its saline vehicle on GDs 10 and 11 at 0500 hr, and re- tion to different agents implicates sex-specific changes on GD 19. Compared to vehicle controls, phenytoin caused fetal body weight loss similarly in all wild-type (+/+) and +/- fetuses (p<0.05). In phenytoin-exposed fetuses, there was a reciprocal p53 gene dose-dependent increase in fetal resorptions in +/- (8%), +/- (18%) and +/- (29%) fetuses, although this trend was not significant. Conversely, loss of p53 alleles resulted in a gene dose-dependent decrease in PPL in +/- (78%), +/- (48%) and +/- (30%) fetuses (p=0.016; p=0.016; p=0.008). The GD 10/11 p53-dependent resorption protection was similar to results with GD 12/13 phenytoin treatment, while the PPL enhancement by p53 was opposite. These results show that p53 differentially modulates phenytoin embryopathies in a gestationally dependent fashion, suggesting different developmental mechanisms. (Support: Canadian Institutes of Health Research)

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

The developing mouse eye is sensitive to many chemical teratogens. Microphthalmia, for example, occurs with intraperitoneal exposure to 2-chlorodeoxyadenosine (2CdA, D8 of gestation) or methylmercury (MeHg, D9 of gestation). Stage dependent differences in the responsiveness of the developing eye to different agents implies sex-specific changes on GD 19. Compared to vehicle controls, phenytoin caused fetal body weight loss similarly in all wild-type (+/+) and +/- fetuses (p<0.05). In phenytoin-exposed fetuses, there was a reciprocal p53 gene dose-dependent increase in fetal resorptions in +/- (8%), +/- (18%) and +/- (29%) fetuses, although this trend was not significant. Conversely, loss of p53 alleles resulted in a gene dose-dependent decrease in PPL in +/- (78%), +/- (48%) and +/- (30%) fetuses (p=0.016; p=0.016; p=0.008). The GD 10/11 p53-dependent resorption protection was similar to results with GD 12/13 phenytoin treatment, while the PPL enhancement by p53 was opposite. These results show that p53 differentially modulates phenytoin embryopathies in a gestationally dependent fashion, suggesting different developmental mechanisms. (Support: Canadian Institutes of Health Research)

133 ALCOHOL DEHYDROGENASE ACTIVITY FROM ZEBRAFISH LIVER AND RECOMBINANT ZEBRAFISH ADH13 PROTEIN.
A.K. Damashapart1,2, H.G. Tomasiewich1, B.C. Lee1 and M.J. Carvan III1,3.
1Great Lakes Water Institute, University of Wisconsin-Milwaukee, Milwaukee, WI, 2Pediatrics, Pharmacology and Taziology, Medical College of Wisconsin, Milwaukee, WI and 3NIHES Marine and Freshwater Biomedical Sciences Center, University of Wisconsin-Milwaukee, Milwaukee, WI.

In continuation of our studies with ethanol toxicity and developmental expression of alcohol dehydrogenase (ADH) mRNA in zebrafish embryos the following studies were undertaken to identify cytosolic alcohol dehydrogenase of adult zebrafish liver that metabolize alcohols to their corresponding aldehydes. Oligonucleotide primers were used to amplify the entire coding region of ADH3 cDNA by reverse transcriptase polymerase chain reaction using total RNA prepared from adult zebrafish AB strain liver. Complete cDNA encoded a 376 amino acid polypeptide that exhibits 93% sequence identity to ADH3 from the marine fish Sparus aurata and 82 and 81% identity to the mouse erythroid and brain, respectively. Zebrafish strains show differential sensitivity to developmental alcohol exposure, and embryos also show differential expression of the ADH3 mRNA. We have expressed zebrafish ADH3, with molecular mass of 40 kDa, in E.coli for analysis of enzyme activity toward ethanol, hexanaldehyde, acetaldehyde, lactic acid, and other substrates. We have also initiated the production of polyclonal antibodies against recombinant zebrafish ADH3 for immunohistochemistry and expression studies. The results will be used in conjunction with mRNA expression data and in situ hybridization analysis to determine the role of ADH3 in the developmental effects of ethanol exposure in zebrafish. Supported primarily by the National Institute on Alcohol Abuse and Alcoholism (NH 8032 AA12480), the University of Wisconsin-Milwaukee, UWM Marine and Freshwater Biomedical Science Center (NIH P30 ES04184) and the Medical College of Wisconsin.

134 IDENTIFICATION AND CHARACTERIZATION OF ALCOHOL DEHYDROGENASE III (ADH11) IN ZEBRAFISH.
M.J. Reimers and R.L. Tanguay, Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO.

Ethanol has been shown to be a teratogen in experimental animals and in humans. The molecular mechanism of ethanol-mediated fetal injury remains unclear. There are two proposed mechanisms of action to explain the teratogenic effects of ethanol. The first mechanism is related to ethanol's ability to directly affect development: and the second requires ethanol metabolism. The focus of this work is on the indirect, or metabolism dependent, processes. We are using zebrafish as a model because their body and physiological characteristics with higher vertebrates including humans and offer many practical advantages. For example, the embryos develop externally, removing the complications of maternal/placental/fetal interactions, and are transparent, providing any developmental stage can be observed without interfering with normal embryogenesis. The goal of these studies is to determine if there is an association between ethanol metabolism and ethanol-mediated developmental toxicity. Here we report the isolation and characterization of a cDNA encoding a zebrafish alcohol dehydrogenase (zeADH). The 1450 bp zeADH is 78%, 72% and 77% similar to the cod fish ADH1, the human ADHII (glu-tathione-dependent formaldehyde dehydrogenase), and to another zebrafish ADHIII clone (GenBank Accession AF359909) at the amino acid level, respectively. To confirm that the zeADHIII has the ability to metabolize ethanol, the protein was overexpressed in baculovirus-infected SF-9 insect cells. For functional studies, the zeADHIII protein was purified to homogeneity by 5-AMP agarose affinity chromatography. Initial kinetics indicate that zeADHIII is significantly more rapid than other substrates. Since this protein has the ability to metabolize ethanol, in situ hybridization experiments were conducted to determine the temporal and spatial expression of zeADH during zebrafish development. These studies indicate that zeADHIII is a suitable model to investigate ethanol-mediated developmental toxicity. This research was supported by NIH/NIAAA grant AA12783.

135 GESTATIONAL AND LACTATIONAL EXPOSURE TO ESTROGENIC CHEMICALS DOES NOT AFFECT MAMMARY GLAND DEVELOPMENT IN FEMALE MICE.
C.J. Fong1, M.R. Fielder2, S.Z. Hadam2 and T.R. Zacharewski1. 1Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI and 2Department of Physiology, Michigan State University, East Lansing, MI.

There exist paradoxical results demonstrating opposite effects of prenatal and postnatal exposure to estrogens on mammary gland development, however, the effects of gestational and lactational exposure to estrogens has not been described. The objective of the study was to assess the effects on mammary gland morphology following gestational and lactational exposure to maternal doses of 0, 0.1, 0.5, 0.5 mg/kg of methylthibuterid (DES) or 0, 0.1, 0.5, 2.5 and 10 mg/kg gestagen (GEN) from gestational day 12 to postnatal (PND) 21. The doses of gestagen approximated human exposure levels. Body weight and anogenital distance (AGD) of female B6D2F1 offspring were measured on PND 7, 21 and 49. Mammary gland whole mounts were examined on PND 49 for epithelial growth (% of fat pad occupied by epithelium), length of mammary epithelial tree, number of terminal end buds (TEBs, undifferentiated proliferating lobule) and density of alveolar buds (ABs, differentiated milk secreting lobules). Prior to analyzing female offspring, an initial study was performed to determine the time course (3, 4, 5, 7 and 10 weeks)
of mammary gland development in native female 36D2F1 mice. Seven weeks of age was chosen as a suitable time point to examine treatment-related changes in mammary development. There were no significant changes in body weight or AGD in female offspring of DES or GEN treated dams. Although there was a trend towards an increase in mammary gland growth and an increased alveolar bud formation in DES-exposed offspring, the results were not significant due to large interlitter variability. We also did not detect any significant changes in mammary growth, TEBs, or ABs in GEN-exposed offspring. These results suggest that combined gestational and lactational exposure to genistein has little effect on mammary gland morphology at human exposure levels.

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

R. C. Liu, J. Y. Rosenblum and R. E. Morrissey. Specialized Toxicology, Schering-Plough Research Institute, Lafayette, NJ.

Pregnant mice received daily s.c. doses of 0.2, 4 and 8 mg/kg on days 6 through postpartum day (PPD) 21 to investigate the effect of TH1-10 on lethality, offspring development, and the survival and reproductive capabilities of F1 generation. Mortality (pregnant and non-pregnant) during the dosing period was 3.9, 12% and 6% in the control, 2.4 and 8 mg/kg dose groups, respectively. The deaths were not associated with abnormal clinical signs, body weights, food consumption or necropsy findings, and the majority occurred after 14 doses which, for the pregnant mice, coincided with the day of parturition. However, the timing also coincided with deaths in non-pregnant mice and male mice in other studies, which suggested that the deaths were unrelated to parturition. Although non-dose-related and perinatal deaths were considered related to TH1-10; a similar pattern of mortality was seen in other studies in which the deaths coincided with the development of acute 11:10 and F1 pup mortality. The increased deaths of 11:10 and F1 pups was not observed in 1:10 mice. The results indicate that, on a relative basis, the early postnatal mortality was significantly lower in the 1:10 mice compared to the normal strain. It was not possible to determine the cause of death in these studies, but it is likely due to a combination of factors, including the effects of maternal and fetal stress. The results suggest that prenatal exposure to TH1-10 may have a negative impact on the survival and reproductive capabilities of the offspring.

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


Lead exposure in gunshot victims with retained lead bullets has been poorly studied, despite more than a century of published case reports and over 50,000 cases each year in the US alone. We studied a series of nearly 300 such patients to determine the time course of blood lead concentration (Pb) for up to six months after injury and to determine the risk factors for elevated Pb. All patients in the study had retained bullets verified by x-ray. We took blood to determine Pb at the time of informed consent (mean: 3.2 days after injury), again when we measured bone lead concentration (PbBone) (mean: 22.4 days), at 90 and 180 days after injury. Geometric mean (range) Pb at admission, 3, 22, 90, and 180 days after admission was 2.0 (0.3-11.8), 3.4 (0.2-23.0), 5.8 (0.3-30.2), 7.3 (0.8-38.6), and 5.5 (1.7-28.4) µg/dl, respectively. Mean (range) PbBone at admission, 3, 22, 90, and 180 days after admission was 5.5 (16.9-26.2) and 9.5 (22.0-39.7) µg/dl. Although only one patient exceeded 10 µg/dl upon admission, 10.2% and 1.8% of patients exceeded 10 and 20 µg/dl at 3 days, 28.4% and 6.8% exceeded these limits at 22 days, 38.3% and 14.8% at 90 days, and 28.9 and 7.9% at 180 days. By 180 days, detectable PbBone was noted in 32 patients. These results indicate that PbBone may be a more sensitive measure of lead exposure than Pb in patients with bone fracture. In our study, elevated PbBone was associated with a higher risk of injury to other organs. The odds ratio of patients with bone fracture exceeding 20 µg/dl during the first 22 days was 8.9 (95% confidence interval 1.7-46.0). Over 22 days, patients with higher calcaneus PbBone and patients over 60 years had higher risk of exceeding 20 µg/dl. Continued surveillance of these patients will reveal the long term time course of PbBone and the factors predicting patients who will exceed national and international norms.

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


To determine the relationship between past (bone lead; PbBone) and present (blood lead; Pb) lead exposure on blood pressure (BP) during pregnancy we analyzed prenatal (3rd trimester) and postnatal (mean=68 days after birth) Pb and BP from over 600 immigrant women, 99% from Latin America. Geometric mean (range) Pb was 2.0 µg/dl (0.4-5.14) and PbBone was 5.2 (0.4-5.14). Postnatal mean (range) PbBone (cortical bone) and calcaneus (trabecular) PbBone (measured by in vivo K-shell x-ray fluorescence) were 8.6 µg/dl (3.3-16.2) and 11.3 µg/dl (3.1-66.4). Mean (range) prenatal and postnatal systolic and diastolic BP was 108.4 mmHg (84-138) and 59.4 mmHg (36-80) and 109.3 (82-136) and 69.7 (53-84). We used structural equation modeling to simultaneously account for the effects of age, body mass index, PbBone, and Pb on BP during and after pregnancy. The model indicated that all lead measures only calcaneus PbBone was associated with BP during pregnancy, and no bone lead measure accounted for maternal BP postpartum. After controlling for the other factors influencing prenatal blood pressure, every increase of 10 µg/dl in calcaneus PbBone is associated with a 0.5 mmHg increase in diastolic blood pressure. The PbBone-BP relationship in non-ethnic minority pregnant women is similar to that found between trabecular PbBone and risk for hypertension in aging women in published work from another laboratory. While the effect is small, it indicates that populations with a wide range of past lead exposure may be at increased risk for elevated blood pressure or hypertension, regardless of present exposure.

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


It is known that there are interindividual variations in the susceptibility to lead (Pb) toxicity. Evidence suggests that the polymorphism at certain genetic loci is known to be related to differentially inherited responses to Pb. Two polymorphic genes have been studied, the vitamin D receptor gene and the &-aminolevulinic acid dehydratase (ALAD) gene. The polymorphism of ALAD gene (ALAD and ALAD) has been associated with differential accumulation and distribution of Pb in blood, bone, and internal organs. Individuals who have at least one copy of the ALAD allele have higher blood Pb (PbBone) and lower zinc protoporphyrin (ZPP) levels however, most of these studies have been done in subjects with high PbBone. We investigated the potential impact of ALAD genotype on Pb toxicokinetic (PbBone and ZPP) in a Mexican adult population from Northern Mexico environmentally exposed to Pb. We conducted a cross-sectional study in 191 adults (both sexes) randomly selected. The ALAD genotyping was based on the PCR method, PbBone were determined by voltammetry and ZPP by hematofluorometry. The ALAD allele frequency in this Mexican population was 5.2% (0.5% ALAD homozygote), relatively lower than the frequency reported in an infant Hispanic population (Astrin et al. 1997). Ann NY Acad Sciences; 514:23-29). Although this study had a selection bias. The average PbBone was 2.0 µg/dl with a range from 1.2 to 37.3 µg/dl. We found no significant differences among participants according to ALAD genotype with respect to PbBone (p=0.244) or ZPP (p=0.123), even after controlling by potential confounders. These preliminary results suggest that ALAD genotype may have no influence on Pb kinetics and toxicity in adults at low Pb-exposure levels. This study was supported by CONACYT-México (Grant 31024-M).

140 SPERM CHROMATIN STRUCTURE IN MEN ENVIRONMENTALLY EXPOSED TO LEAD.


The evaluation of sperm chromatin is relevant since chromatin abnormalities may be associated with reproductive failures and offspring development. Despite the condensed structure of human sperm chromatin, it can be damaged by exposure to
toxic agents. Lead (Pb) exposure is still a public health concern in developing countries, which detrimental effects on male reproduction are not well studied. Few studies have reported Pb effects on sperm chromatin; human occupationally Pb-exposed have levels of acid-citrate resistant sperms, but little is known about Pb effects at low exposure levels. Therefore, we evaluated sperm chromatin structure (SCSA) in 66 semen samples from men environmentally exposed to Pb (blood Pb levels of 10.3μg/dL) by the SDSA-descondensation technique (SDS-ncr) and the sperm chromatin structure assay (SCSA) by flow cytometry. Results were correlated with Pb and zinc (Zn) levels in semen compartment determined by atomic absorption spectroscopy. SDS-ncr showed that chromatin condensation significantly increased as Zn in spermatozoa (ZnSp2), in seminal liquid (ZnSL), and in seminal liquid (PsBLs) increased. From the SCSA parameters, only green fluorescence (GRN, accessibility to dyes) negatively correlated with SDS-ncr. Alpha+ positively correlated with ZnSp2 and ZnSL, whereas Pb did not. Co-treat (DNA denaturation) did not correlate with Pb nor Pb in any compartment. GRN negatively correlated with ZnSp2 and ZnSL (p<0.05), but not with PsBLs (p=0.144). In addition, positive correlations were observed between ZnSp2 and ZnSL with PsBLs. Taken together, these results suggest the increase in chromatin condensation observed with Zn may be an indirect effect of Pb. Thus, these results support that the exposure to low Pb levels alters sperm chromatin integrity, but does not damage sperm DNA. Further studies in larger populations are needed to confirm the indirect effect of Pb. This study was supported by CONACYT-Mexico (Grant 31024-M).

143 PYRROLOGINES QUINOLONE AS A NOVEL THERAPEUTIC CHELATING AGENT TO REDUCE TISSUE LEAD LEVELS AND TOXICITY.

J. Sheridan1, D. Rejmanek1, J. Lofius1, J. Mah1, J. Rosen1 and D. Smith1
1. Environmental Toxicology. University of California Santa Cruz, Santa Cruz, CA. 3. University of Southern California, Los Angeles, CA and 1. Memorial Medical Center, Bronx, NY.

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

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

D. R. Smith1, M. Hernandez-Avila1, M. Teller-Rojas1, A. Mercado1, H. Hu1, S. Gomez1 and R. Gwiazda1
1. University of California, Santa Cruz, CA. 2. INSEP, Guanajuato, Mexico and 3. Harvard, Boston, MA.

Studies have suggested that plasma Pb levels may better reflect the toxicologically labile fraction of circulatory Pb that is more freely available for exchange with target tissues than does whole blood Pb levels. Here, we conducted a longitudinal assessment of the relationship between Pb in whole blood and plasma in environmentally exposed reproductive age women (n=63) living in Mexico City, Mexico. Samples were collected using trace metal clean techniques and analyzed for Pb using HR-ICP-MS. A subset of subjects provided repeated blood samples weekly for 4 weeks (n=17 subjects), or every 1 to 2 months over 9 months (n=14 subjects). Plasma Pb concentration was significantly positively associated with whole blood Pb in a curvilinear fashion over the range of blood Pb values observed here (2.13 - 39.7 mg/dL). This relationship was best described by the function: Plasma Pb = 1.2392 - 0.00898* Pb blood, where SEcoefficient = 0.0054, SEconstant = 0.063 (n=63 subjects, n=141 observations). Results from the short and long-term repeated collection subjects indicated that the within and between subjects components were not significantly different between the two subsets of subjects. The between subjects component accounts for 78% of the variance in plasma Pb levels, while the residual variance (22%) may be attributed to other unmeasured factors. Analysis of the %plasma Pb vs blood Pb relationship at blood Pb levels <100μg/dL suggests that relationship is neither constant or linear. This study demonstrates that plasma Pb measurements may be applied to general clinical settings, provided that established trace metal clean techniques are adopted. They also show that the relative (% partitioning of whole blood Pb in plasma naturally varies by a factor of -2 to 4-fold between subjects at a given blood Pb level. Overall, these data indicate that plasma Pb may provide a more meaningful measure of toxicologically available Pb that may complement whole blood Pb level measurements.

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

R. Gwiazda1, K. Sauls1, M. McDermid1 and D. Smith1
1. Environmental Toxicology. University of California Santa Cruz, Santa Cruz, CA. 3. Dept of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD and 1. Dept of Medicine, University of Maryland, Baltimore, MD.

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

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

M. H. Whittaker, M. Lipsky, G. Wang, X. Chen and B. A. Fowlers, Epidemiology and Preventive Medicine, The University of Maryland, Baltimore, MD.

Male Sprague-Dawley rats were administered concentrations of 25, 50, or 100 ppm lead (as lead acetate) in deionized drinking water for 30, 90, or 180 days. At the 180 day timepoint, kidney/body weight ratios were 70%, 75%, and 79% of the control value, and kidney aminolevulinic acid dehydratase (ALAD) levels were 53%, 33%, and 33% of the control value in the 25, 50, and 100 ppm dose groups. Decreased kidney/body weight ratios at 180 days were attributed to increased terminal body weights, which ranged from 130-138% that of the control group. Significant changes in other variables were observed, but only at the 30 and 90 day timepoints. Decreases in blood ALAD and increases in urinary protein were observed after 90 days of lead exposure. At 90 days, blood ALAD levels were 59%, 54%, and 57% of that of the control, in the 50, 25 and 100 ppm dose groups. At this same timepoint, urinary protein was 13%, 15%, and 16% of that of the control. These data suggest that some adaptation/compensation took place because these changes were not observed at 180 days. Pathological analyses of kidney sections showed usual morphologic alterations associated with lead exposure. Compared to control animals, a larger number of mitotic figures were observed in proximal tubule cells in test animals in all dose groups after only 30 days of lead exposure. Karyomegaly along with coarsen nuclear inclusions in enlarged atypical nuclei were observed in proximal tubule cells at 100 ppm in test animals. At 90 days, karyomegaly with inclusion bodies was observed in the 100 ppm group. At 180 days, karyomegaly with inclusion bodies was observed in the 50 and 100 ppm dose groups. Cells with karyomegaly were primarily from the P3 segment. Based on changes observed at all lead concentrations, a LOEL of 25 ppm was assigned. This LOEL is currently being used in a six-month metal/metalloid mixture study. (Supported by USEPA Star Grant R827161-01-0).

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

M. B. Justard, T. P. Nunnappakam, K. S. Bailey, K. A. Graves and K. Smith, College of Pharmacy, Xavier University, New Orleans, LA.

The present study was designed to determine if manganese exposure caused species specific responses in rodents. CD-1 Mice and Sprague-Dawley rats were investigated for alterations in reproductive parameters after administration of manganese acetate by oral gavage. Reproductive toxicity produced in mice occurred at much lower doses than in rats. The dose-ranged of 50 mg/kg was determined to be toxic for mice, whereas 2500 mg/kg was toxic to rats. Rats were dosed with a sublethal concentration of 76.5, 153, 306 mgMn/kg for 63 days. Mice were dosed with 7.5, 15 and 30 mg Mn/kg for 43 days. Cross-mating was achieved between the high dose of animals of each sex with controls of opposite sex, and others were mated with their respective groups. After a 5-day cohabitation period with females, the males were separated. On day 18 of gestation, necropsy was performed and a comprehensive examination was made of reproductive organs. Other end points observed included uterine implantation sites, resorptions, fetus number, fetus weight and the corpora lutea. Differences in pregnancy rates, fetal weights, fetal number, resorptions, and number of corpora lutea were observed between the control groups and the manganese acetate treated groups in both rats and mice. The results of the present study demonstrate that mice are more sensitive to manganese as compared to rats. In both the species, control females mated with high dose males had fewer pregnancy rates, and males were affected more than the females. Our study suggests that the pattern of response was same in both species even though it occurred at different concentrations. As a result, mice and rats may both be a valid model of toxicity testing, even though differences between the rodent exist. Supported by ATSDR US05/AT398948-10.

INDUCTION OF HEPATIC METALLOTHIONEINE BY MANGANESE IS MEDIATED BY INTERLEUKIN-6.

S. Himeo1, K. Kobayashi2, M. Sato3, C. Togutsu4, T. Hasegawa5, Y. Seko6 and N. Isuzu7, 1Kakusei University, Japan, 2Kioi Pharmaceutical Co., Ltd., Nagano, Japan, 3NIES, Tsukuba, Japan and 4Yamashita Institute of Environmental Sciences, Fuiyishida, Japan.

Inducers of metallothionein (MT) include metals, cytokines, hormones and other physical and chemical stressors. Induction of MT by metals such as Zn has been shown to be mediated by a specific transcription factor, MTF1. However, the mechanism of MT induction by Mn has been poorly elucidated. Therefore, we investigated the characteristics of Mn-induced MT and the mechanism of MT induction by Mn. Male ICR mice were injected subcutaneously with MnCl2 and sacrificed 24 h after the injection. MT concentration was increased dose-dependently in the liver by MnCl2 administration, but not in the kidney. The increases in MT concentration were most evident in the liver, serum interleukin-6 (IL-6) concentration reached the peak at 6 h after the injection, and serum albumin A1, the indicator of hepatic inflammation, gradually increased. However, serum IL-1β, TNF-α, GPT or BUN did not increase after the MnCl2 injection. To confirm the involvement of IL-6 in MT induction by MnCl2, IL-6 null and control mice were injected with MnCl2. In IL-6 null mice, no increase in hepatic MT or serum albumin A1 was observed. These data suggest that the induction of hepatic MT in mice by MnCl2 administration is entirely dependent on the production of inflammatory cytokine, IL-6.

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

T. P. Nunnappakam, K. S. Bailey, D. Magee, K. A. Graves and M. B. Justard, College of Pharmacy, Xavier University, New Orleans, LA.

Manganese plays a pivotal role as a ubiquitous constituent in the environment that is beneficial or essential at low intake levels while inhalation or oral exposure to high levels can cause adverse effects. In the present study, the effect of manganese on reproductive and teratological function in Sprague-Dawley rats was investigated. Oral administration of manganese acetate was given daily to male and female Sprague-Dawley rats at 0(control/control positive), 76, 156 and 306 mgMn/kg body weight for a period of 63 days prior to mating. 5 days during mating and 17 days of pregnancy until necropsy. At necropsy, the end points of target organs were examined for treated males included: organ weights, percent sperm motility, sperm concentration in testis and caudal duct. Crossover mating trials were accomplished between high dose animals of each sex and control rats of opposite sex. Results of the crossover were compared to mating within control positive groups to determine which sex and dose group were affected. End points for females included: organ weights, fertility assessments, total corpora lutea, and resorptions. Our data results demonstrated that there was a decrease in organ weight in both sexes. There was a significant reduction in testicular and caudal sperm counts in all groups. Sperm motility was reduced in all groups, but was significant in rats dosed at 306 mgMn/kg. Fetal observations showed lower values in pregnancy rates, uterine weight, and the number and weight of fetuses. Control positive females mated with control positive males had a pregnancy rate of 78.5% and the average resorption rate of 0.33/litter. The pregnancy rate was 60% and an average resorption rate of 1.33/litter in control females mated with treated males. Treated females mated with control males had a pregnancy rate of 75% and resorption of 1.08/litter. This study demonstrated that manganese damages spermatogenesis and fertility profile of both male and female rats, but comparatively, male rats are affected more than females. Supported by ATSDR US05/AT398948-10.

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


Hexavalent Chromium (Cr VI) is a well-established human carcinogen and exposure to it is widespread. Cr VI induces genotoxicity and cell cycle arrest, but the specific mechanisms involved are uncertain. Most Cr VI studies have focused on
Chinese hamster ovary cells (CHO) or human tumor lines such as A549 cells. But these are known to have altered cell cycle machinery and may not be suitable models for such mechanistic investigations. We therefore replaced the cytotoxicity of Cr(VI) with these lines and in primary human bronchial fibroblasts (PHBF). We found that Na2CrO4 (NC) was cytotoxic to PHBF, inducing 79, 59, and 18% and 0 percent survival at 1, 2.5, 5, and 10 μM, respectively, but contrast NC was less toxic to A549 cells inducing 68, 68, and 22.3% percent survival at 2.5, 5, and 10 μM, respectively. CHO cells were much more similar to PHBF with NC inducing 79, 69, 35, and 0 percent survival at 1, 2.5, 5, and 10 μM, respectively. We also found NC induced cell cycle arrest (expressed as mitotic index) in PHBF. Specifically, in PHBF, the mitotic index was 44, 46, 26 and 2% at 1, 2.5, 5 and 10 μM, respectively. There was much less arrest in A549 cells, as the mitotic index did not change significantly with dose (5%, 5%, 3%, 1% and 0 μM, respectively). Similarly, CHO survival was more sensitive than PHBF to NC-induced arrest at low doses (78% and 69% at 0.5 M M and 1 μM, respectively), but were similar at the two highest doses (19% and 4% at 5 and 10 μM, respectively). In conclusion, CHO cells and A549 cells appear to be inadequate models for investigating the mechanisms of cytotoxicity and cell cycle effects of Cr(VI). Therefore, in primary human New human bronchial cell cultures are clearly needed to allow for mechanistic investigations of the cell cycle effects of Cr(VI) and perhaps other met. Future efforts in our laboratory are aimed at assessing the utility of CHO and A549 in evaluating Cr(VI) genotoxicity and developing new human bronchial cell culture models.

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

Epidemiology and Public Health, Yale University, New Haven, CT and Bioengineering, University of Washington, Seattle, WA.

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

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

K. A. O'Hart, L. R. Klei and A. Bachowsky. Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.

Inhaled hexavalent chromium Cr(VI) can promote fibroproliferative disease, airway hypersensitivity, and possibly lung cancer through poorly defined mechanisms. Therefore, cultured epithelial cells were used to examine the hypothesis that non-toxic Cr(VI) exposures activate gene transcription through specific oxidant-sensitive signaling pathways. These studies demonstrated that 1-10 μM Cr(VI) induced increased reactive oxygen species and selectively activated JNK, relative to ERK or p38 MAP kinase. Stimulation of JNK by these levels of Cr(VI) was comparable to the effects of 1.0 mN M H2O2. However, in contrast to H2O2, 10 μM Cr(VI) was only marginally toxic. JNK activation was blocked by pre-incubating the cells with either N-acetylglucosamine or PGE2, a selective inhibitor of the Src family of tyrosine kinases (SFKs). Treatment with tropine to block mitochondrial respiration or with exogenous catalase only partially attenuated Cr(VI) induced JNK activity. Neither Cr(VI) nor H2O2 activated JNK in SFK deficient cells. H2O2, but not Cr(VI) activated JNK in cells containing only Src. Transient transfection with wild type or mutant p120cbl indicated that both Cr(VI) and H2O2 require this adapter protein.
156 ADAPTIVE ANTIOXIDANT RESPONSES IN HUMAN A549 LUNG CELLS TREATED WITH HEXAVALENT CHROMIUM.


Hexavalent chromium (CrVI) is genotoxic and has been classified by the International Agency for Research on Cancer as a Group 1 carcinogen. CrVI is reduced intracellularly to CrIII involving the production of various intermediate species, including CrV and CrIV and accompanying the production of reactive oxygen species (ROS). The resulting oxidative stress is believed to be responsible for many of the cellular effects caused by CrVI and may contribute to changes in gene expression. In the current study, CrVI-mediated changes in the expression of several stress-related genes were investigated in human A549 lung cells. In association with oxidative DNA damage, sodium dichromate gave a dose-dependent increase in 3,4-fold increase; 100μM in antioxidant capacity of cells at 2h as measured by the inhibition of pholasin oxidation and bioluminescence (ABEI assay; Knight Scientific Ltd., ). There was no decline in intracellular ATP concentrations. A persistent increase in intracellular oxidative stress (84, 344) and a transient elevation of cytosolic thiorodoxin (TRX) (1.8-fold increase at 2h as measured by densitometry) protein levels was also seen by western blotting. Nuclear protein assays confirmed the elevation of HO-1 mRNA but also demonstrated that the mRNA for various other antioxidant systems (glutathione reductase, Mn-superoxide dismutase and NADPH:quinone oxidoreductase) along with the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was not elevated. In conclusion, in association with DNA damage, treatment with sodium dichromate appears to elevate selective antioxidant defenses that may contribute to protective adaptation to exposure. (Sponsored by the Coli Foundation).

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

L. Ogden, T. Graham, M. Mahboob, A. Atkinson, M. Hamersley and N. Sarkar. Biomedical Sciences, Teagge University, Teagge, AL; Sponsor: R. Dashi.

The effects of low doses of zinc chloride (ZnCl2) on the reproductive performance have not been well described. The major objective was to determine the effects of low doses of ZnCl2 on reproductive performance in a mammalian species. Life stage evaluated were puberty, maturation, pregnancy, gestation, and lactation. Both males and females were exposed to either 0.00, 0.78, 1.56, or 3.125 mg/kg ZnCl2 daily by oral gavage until they were terminated. Males were sacrificed after cohabitation and females after lactation. Reproductive parameters evaluated were mean litter size, pup viability index, pup litter weight, live births, clinical signs, fertility index, post partum dam body weights, food consumption and male and female body weights. Abnormal nursing and nesting behaviors were observed in the mid and high dose groups. This was characterized by hyperactivity, absence of nest building, failure to allow pups to nurse and killing pups. There were no significant differences in the F1 male and female body weight gain or food consumption. There was a dose dependent trend toward decreased implantation efficiency. Litter size was greatest in the control group and decreased as dose increased. There was a trend toward a decrease in the number of live births in the mid and high dose groups. Mortalities occurred in all treatment groups but were greatest in the low group, high group and the mid dose groups, respectively. These data suggest that zinc adversely affects some reproductive parameters when mice were exposed to oral doses as low as 0.78 mg/kg. (Supported by MH/PA/ATSDR Cooperative Agreement # U50/ATU3922600009).

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

S. Park*, S. H. Garrett*, M. A. Sens*, S. Sonnij* and D. A. Sens*. *Urology, West Virginia University, Morgantown, WV and Pathology, West Virginia University, Morgantown, WV.

The third isomer of metallothionein (MT-3) has a limited tissue distribution, being confined primarily to neural tissue and the kidney; however, overexpression of this protein is found in many cancers of the breast, prostate and bladder.

Overexpression of MT-3 has been demonstrated in bladder tumors with the levels correlating to the type and grade of the tumor. whereas MT-3 expression in the normal human bladder was undetectable. Recently a new normal bladder cell line, UROSA, was characterized as a useful in vitro model system of human urothelium and found not to express MT-3. The consequence of MT-3 overexpression in urothelial cell lines was evaluated by stable transfection with MT-3 under control of the CMV promoter. Twenty clones were isolated and analyzed for the expression of MT-3. RT-PCR confirmed the expression with products being identified at 25 cycles of PCR. Similar amounts of PCR products were obtained using primers for gyrasealdehyde 3-phosphate dehydrogenase indicating that the level of MT-3 expression was similar to that of a human kidney MT-3 clone. The levels of MT-3 protein were surprisingly low, however, with levels often pushing the limit of detection (0.5 ng/μg protein). Since MT-3 has been successfully overexpressed in many cell lines of the prostate, kidney, and breast, the nearly non-existent levels of MT-3 in the urothelial cell line suggested that there was the potential for selection against MT-3 expression. MT-3 was overexpressed in the regulatable vector system, pLZIP.CMV, in which stable transformants can be selected under MT-3-dormant conditions and then induced with the exogenous agent isopropl β-D-thiogalactopyranoside (IPTG). Using this vector system, levels of MT-3 protein were significantly higher (average 4.13 ng/mg protein).

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

V. Guad* , S. H. Garrett*, S. Sonnij*, M. A. Sens*, D. A. Sens*. *Urology, West Virginia University, Morgantown, WV and Pathology, West Virginia University, Morgantown, WV.

MT-3 is a unique among the metallothionein gene family in that its expression in normal cells is limited and it has a neuronal growth inhibitory activity. The present study was designed to determine if overexpression of MT-3 and 1E influences the growth and chemotherapeutic drug resistance of breast cancer cells. The cell lines MCF-7, T-47D, Hs 578T and MCF-10A were stably transfected to overexpress the MT-3 or MT-3 1E genes using the pcDNA3.1/Hygro (+) vector. Overexpression of the genes was confirmed in 5 independent clones by a combination of RT-PCR and immuno blot analysis. Growth and chemotherapeutic drug resistance were determined by cell counts and colony formation assays. Overexpression of MT-3 was demonstrated to inhibit the growth rate of the breast cancer cell lines by approximately 50% compared to vector-only controls (p<0.001 for each comparison) but to have no effect on the growth rate of non tumorigenic MCF-10A breast cells. For all cell lines, the overexpression of MT-3 resulted in increased resistance to: Cd2+, cisplatin, doxorubicin, paclitaxel and methotrexate. Overexpression of the 1E isoform conferred a resistance to the above chemotherapeutic agents, but did not cause an inhibition of cell growth.

160 STIMULATION OF CYTOKINE PRODUCTION IN MACROPHAGES BY ZINC Y26 METALLOTHIONEIN.


We previously reported that human cytomegalovirus major immediate-early promoter activity is controlled by intracellular zinc levels via metallothionein (MT)- mediated regulation of NF-kappa B activity (Ann. Rev. Biomed. Eng. 4, 207-214, 2000; Toxicol. Appl. Pharmacol. 173, 143-153, 2001). This transcriptional factor is an important protein involved in expression of several cytokine genes and regulation of various cell functions. The expression of MT-3 is tightly regulated in many kinds of toxicological responses. In the present study, we evaluate the effects of MT on the expression of proinflammatory cytokines in macrophages. We used peritoneal exudate macrophages (PEMs) obtained from MT-/- or MT+/- mice. Secretion of tumor necrosis factor (TNF) stimulated by lipopolysaccharide (LPS) from MT-/-PEMs was significantly less than in wild-type control. LPS-stimulated expression of TNF-alpha was decreased in MT-/-PEMs. In electrophoretic mobility shift assays, LPS-stimulated activation of the DNA-binding activity of NF-kappa B is also decreased in MT-/-PEMs. in vivo response was also evaluated in cell-free ascites fluid harvested 1 h after ip injection of LPS. The response of PEMs in MT-/- mice was less than in wild-type mice in vivo in the same manner as in vitro. These findings suggest that the LPS-induced production of TNF in mouse peritoneal macrophages is upregulated by MT through the modulation of NF-kappa B activity. Expression of other inflammatory cytokines, such as interleukin (IL)-1alpha and IL-6, is also downregulated in MT-/-PEMs. The function of PEMs was changed by MT deficiency in terms of LPS-induced cytokine responses. Thus, MT plays an important role in the LPS-induced activation of PEMs. Since MTis induced by various xenobiotics, it is possible that some chemicals modulate host defense system through the induction of MT.
THE ROLE OF METALLOTHIONEIN IN APOPTOSIS INDUCED BY ZINC IONOPHORE.

M. Takiguchi, E. Tasaki, M. Konoh, M. Higashimoto and M. Sato. Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Various cellular functions are influenced by essential trace elements such as the divalent cation zinc. It has been shown that exclusively released zinc induces neuronal cell death associated with apoptosis. The metal binding protein, metallothionein (MT), is probably involved in zinc homeostasis since it typically binds large amounts of zinc. Recent work has provided new role of MT in regulation of cell cycle and apoptosis. In this study, we investigated that excessive zinc accumulation in fibroblast cells induces apoptosis and necrosis depending on zinc concentration, and MT inhibits the apoptosis induced by zinc. Intracellular zinc concentrations in MT-I and MT-II null (MT-I-/), and wild-type (MT-I+/+) fibroblast cells exposed with ZnCl₂ for 2hours were not different from non-treated cells. The data indicate that the physiological concentration of zinc in cells is strictly controlled. Then we used Na pyrithione, zinc ionophore, and it increased the zinc concentration at 2.5 fold compared to the control, and co-existence of ZnCl₂ and Na pyrithione in the cell culture medium further increased the zinc concentration (7.5 fold) in both cells. MT-I/- were more susceptible to Na pyrithione than MT-I+/+ cells as assessed by MTT. But sensitivity of co-exposure with ZnCl₂ and Na pyrithione was not different between MT-I- and MT-I+ cells. DNA fragmentation, one of the hallmarks of apoptosis, was induced by Na pyrithione, although co-treatment of ZnCl₂ and Na pyrithione did not induce DNA fragmentation. The DNA fragmentation by Na pyrithione in MT-I- cells was stronger than that in MT-I+ cells. These results indicate that the concentration of zinc in cells determines mode of zinc-induced cell death. MT inhibits the apoptosis induced by zinc.

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


The effects of low doses of zinc chloride (ZnCl₂) on the reproductive performance have not been well described. The major objective of this study was to determine the effects of low doses of ZnCl₂ on the reproductive performance in a mammalian species. A breeding trial was conducted where males and females were divided into four groups and exposed to 0.00, 0.28, 1.56, or 3.125 mg/kg of ZnCl₂ per day for 10 weeks. The major objective was to determine the effects of low doses of ZnCl₂ on clinical pathology parameters that may adversely influence reproductive performance. The hematological parameters included WBC, RBC, HGB, HCT, MCV, MCH and MCHC. The clinical chemistry values included, Na, K, Cl, PO₄, Ca, ALT, ALB, ALBUNENT, GLOBULIN, Total Protein, Amylase, Creatinine, CK, BUN, and GLUCOSE. Blood samples were collected at scheduled sacrifices (post copulation for males and post lactation for females). The most relevant changes in hematological parameters were seen in males. High dose males had decreased MCH and MCV. Although not significant, there were trends toward increased WBC counts in all ZnCl₂ treatment groups. Few significant changes were seen in the clinical chemistry in either sex. However, creatinine was elevated in high dose males and CK was elevated in high and mid dose males. In summary, oral exposure to ZnCl₂ at the levels in this experiment did not alter hematological or clinical chemistry parameters to the degree necessary to influence reproductive performance. (Supported by MHSIP/ATSDR Cooperative Agreement # U50/ATU3922600009).

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


Trace amounts of zinc are required for functional activity of several biological molecules; however, excessive amounts may be toxic to mammals. This study was conducted to determine if low levels of zinc chloride (ZnCl₂) given to F₁ generation affects antioxidant enzyme activity in the brain, testis, liver and kidney of the F₂ offspring. CD-1 males (70 days) and females (99 days) were exposed to 0.000 (control), 0.78 (low), 1.560 (mid), and 3.125 (high) mg/kg/day of ZnCl₂ via oral gavage. After weaning, F₁ males were weighed and sacrificed. Brain, testes, liver and kidneys were removed and assayed for the catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities. In the brain, the SOD activity increased, while the GPX and CAT activities decreased in all treatment groups. Liver SOD and GPX activities increased in all treated groups, except at the low dose, in which the GPX activity decreased. CAT activity decreased in low and high dose and increased at the mid dose. The kidney SOD activity increased in all treated groups.

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

K. D. Welch, T. Z. Davis and S. D. Aust. Chemistry and Biochemistry, Utah State University, Logan, UT.

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

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

M. L. Chiarappa-Zucca, R. C. Finkel, J. E. McAninch, R. E. Mantinelli and K. W. Turcklaus. Lawrence Livermore National Laboratory, Livermore, CA and *Thermawave, Fremont, CA.

A method using accelerator mass spectrometry (AMS) was developed to measure low levels (femtomoles) of beryllium in biological samples. This method provides the sensitivity to investigate macromolecular complexes formed with beryllium at low exposure doses and will provide further understanding about the molecular targets involved in beryllium disease. Berylliosis is a debilitating, progressive and potentially fatal lung disease that develops in individuals exposed to beryllium. Proof of the method was tested by administering 0.05, 0.5 and 5.0 μg 9Be and 10Be by IP injection to 30 g male ICR mice. The mice were euthanized after 24 h and blood, femurs, feces, urine, kidneys, spleen, liver, thymus and lung were prepared for AMS analysis by acid digestion. Highest levels of Be were found in the liver and the spleen (6.0 and 2.0% of whole mouse dose, respectively) while the lowest levels were found in blood, lung and thymus. Beryllium levels were dose-dependent in the spleen and liver. The detection limit of Be in tissue by this method is approxi- mately 2 amol and the analysis is linear over 2 orders of magnitude. Possible sample size effects for measuring Be by AMS showed that similar results were obtained when using samples that were between 3.0 and 150 mg of dosed liver tissue. Precision of 8 replicates of pooled liver tissue was 5% while the variability between 20 pooled livers was 10%. These results show that routine quantification of atto- femtomole levels of Be in tissues is possible. This method should enable further studies to understand the molecular dosimetry and mechanisms of Be toxicity in biologic studies. This work performed under the auspices of the USDOE by LLNL (W-7405-ENG-48) with support from the USDOE/DEER.

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


Metals have become a problem in some countries(1). A less studied metal is vanadium - a highly oxidant metal- which could be an essential or a toxic element. One of the vanadium sources in the air is the combustion of fossil fuel products.
Mexican Maya oil has one of the highest V concentrations. Mexico City with three million circulating vehicles, has an increase in V over a thirty years period in measured in human lungs. This information made us to investigate the induction of lipid peroxidation in four organs (lung, testes, liver and kidney) in a mice model after the inhalation of V2O5. Methodology: CD-1 mice (18h) 0.01M or 0.02M during 4 weeks twice a week. Controls inhalate only the vehicle. Mice were sacrificed after the first inhalation, and each week. Peroxides were quantified by light spectrometry using a K-assy: 1PO-CC. Results: Lung and testes control mice reported the lowest basal concentrations, statistically significant increase was observed in testes at 0.01M. Kidney and liver showed an increase in lipid peroxides in a dose-dependent manner. Liver at 0.02M reported the highest lipid peroxidation concentrations. Conclusions: The inhalation of V induced lipid peroxidation in the liver and the kidney, with no differences related with the dose. Lung and testes reported almost no peroxidative activity as a consequence of V inhalation. These differences might be related with the organ content of antioxidant agents, such as glutathione, the ability to concentrate the metal, or other specific physiologic characteristics of the organs which requires further analysis.

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

167 GENOTOXICITY STUDIES OF TWO HISTORICAL SAMPLES OF NICKEL REFINERY DUST.
F.M. Clements and J.R. Lambeth. Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA.
The INCO nickel refinery in Clyde, Whales, U.K., was actively in operation from 1901 until 1948. Since 1901, there have been a total of 365 cases of cancer reported in the workers of this refinery, including 85 nasal cancers and 280 lung cancers. From 1901 to 1923, the incidence of these cancers was very high. The INCO refinery then changed the process of nickel refining after 1923 (Draper, 1994, ETP). In the change in the refining process eliminated the oreclitic component. The incidences of cancers were subsequently reported to be greatly reduced from 1925-1930. A refinery dust sample was taken in 1920 and another in 1929. Both samples contain primarily NiO. The main difference between the two dust samples is the presence of a nickel arsenide (Ore-Ore) in the 1920 sample. The arsenic content in the 1920 sample is 10% and in the 1929 sample is only 1%. We hypothesized that it is the nickel arsenide component of the 1920 sample that was responsible for the nasal and respiratory cancers in the refinery workers. A pure sample of the nickel arsenide (oreclitic) was obtained and used for comparison. Our results show that all of the samples were taken up by the cells by phagocytosis at similar rates, both the 1920 and the 1929 samples, and the pure oreclitic sample are cytotoxic to 10T1/2 mouse embryo cells. The LC50 values are (3.2 ± 0.7)μg/ml for the 1920 sample, (1.9 ± 0.2)μg/ml for the 1929 sample, and (2.5 ± 0.3)μg/ml for the pure sample of oreclitic. In support of our hypothesis, we found that the 1920 sample is able to induce morphological transformation in a dose-dependent manner, with 9 foci per 20 dishes treated with the highest concentration of 7.5μg/ml. We found that the 1929 sample did not induce morphological transformation. Therefore, the data provides evidence that the 1920 sample has carcinogenic potential, consistent with induction of nasal and respiratory cancers in the refinery workers before 1923. Further investigation is in progress with the pure sample of oreclitic to determine its transforming ability.

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

169 SELECTION OF FILTER MATERIAL FOR METAL COMPOSITION ANALYSES OF AMBIENT PARTICULATE MATTER SAMPLES BY ED-XRF.
P.B. Maciejczyk and L.C. Chern. Environmental Medicine, NYU School of Medicine, New York, NY.
Energy-dispersive X-ray Fluorescence (ED-XRF) is becoming a popular chemical analysis technique in large-scale air sampling campaigns. Filters or substrates used to collect ambient particulate matter (PM) for XRF analysis should have the following characteristics: (1) low mass to reduce scattering of incident radiation thus minimizing background count, (2) low concentrations of elements to be measured, and (3) low penetration of particles below the surface. Five types of commercially available filter material were analyzed for background metal content using a JVAR EX-6600 AF instrument. These filters were quartz fiber, Whatman paper, Teflon on polyolefinic ring support, Teflon on fiber support, and polycarbonate membrane. A sample of results is shown in Table 1. Quartz fiber filters were unacceptable for PM metal analysis due to high background metal concentrations and high penetration factor. Additionally, light elements such as sodium, magnesium, and aluminum could not be analyzed due to high silicon concentration. Whatman paper also had high metal concentrations in blanks although much lower than quartz fiber. Filter materials that have been found most suitable were the thin membrane type: Teflon and polycarbonate, as they have lowest background and penetration. Caution should be exercised while sampling on Teflon with fiber support as sampling on the support, i.e., fiber side of the filter, is a common mistake. Supported in part by EPA R827510. TABLE 1: Selected background metal concentrations in filter material (μg/cm²).

<table>
<thead>
<tr>
<th>Material</th>
<th>Si</th>
<th>V</th>
<th>Fe</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz fiber</td>
<td>104</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td>126</td>
</tr>
<tr>
<td>Whatman paper</td>
<td>200</td>
<td>0.77</td>
<td>100</td>
<td>85</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Teflon on polyolefinic ring</td>
<td>15.7</td>
<td>1.8</td>
<td>9.7</td>
<td>0.7</td>
<td>2.3</td>
<td>7.6</td>
</tr>
<tr>
<td>Teflon on fiber support</td>
<td>16.6</td>
<td>3.3</td>
<td>19.2</td>
<td>2.3</td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>15.7</td>
<td>1.8</td>
<td>9.3</td>
<td>2.7</td>
<td>7.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

170 THE EFFECTS OF AN ORGANOPHOSPHATE (OP)-CARBAMATE (CB) PESTICIDE MIXTURE ON CORE TEMPERATURE AND MOTOR ACTIVITY IN THE RAT.
Pesticide risk assessment has traditionally been based on the toxicological response to single agents. Dose-additivity has been the default in risk assessment evaluations of pesticides with a common mechanism of action, but there could be supra-additive or infra-additive interactions of pesticide mixtures. We have found that rats exposed to anticholinesterase OP or CB pesticides display a transient hyperthermia.
followed by a delayed fever. Thus, the hypothesis of additivity of an OP/CAR mixture may be assessed using thermoregulatory endpoints. A fixed-ratio ray design was developed from single chemical dose-responses to assess the effects of a mixture of the OP and the carbamate insecticide chlorpyrifos (CAR). Adult, male Long Evans rats were surgically implanted with radio transmitters (Data Sciences) to monitor core temperature (Tc) and motor activity (MA). After recording a 24 hr baseline of Tc and MA, rats were dosed orally with corn oil, 30 mg/kg CHP, 75 mg/kg CAR, or one of the following 2:1 mixtures of CHP:CAR: 4.7:2.3, 5.3:4.7, 14.0:7.0, 18.7:9.3, or 23.3:11.7 mg/kg. Exposure to pure CHP and CAR yielded an acute, marked reduction in Tc followed by a delayed fever for at least one day post dose. The hypothalamic response to the binary mixtures was diminished compared to the pure chemicals. A mixture of 23:3:17.7 mg/kg led to a hypothalamic response half that of 30 mg/kg CHP (assuming dose-additivity, the hypothalamic response of these two treatments should be equal). However, the CHP:CAR mixture <14.6:7.0 mg/kg led to a delayed fever similar to that elicited by pure CHP. MA post dosing was reduced in the 30 mg/kg CHP group, whereas the mixtures did not affect MA. Overall, there is an infra-additive response to a mixture of CHP:CAR when assessed by the hypothalamic response. This response is mediated through stimulation of cholinergic thermoregulatory pathways. The delayed fever mediated by paroxysmal cholinergic systems, is unaffected by the CHP:CAR mixture. This abstract does not necessarily reflect USEPA policy.

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

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

172 DEVELOPMENT OF A RESPONSE SURFACE FOR THE TOXICOLOGICAL INTERACTION BETWEEN CHLORPYRIFOS AND PARATHION USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING.
H. A. El-Mari1, M. Y. Yushak and M. M. Murrza. 1-Computation Toxicology Laboratory, Division of Toxicology, Agency for Toxic Substances and Disease Registry, Atlanta, GA and Rollins School of Public Health, Emory University, Atlanta, GA. Sponsor: M. Murrza.

Chlorpyrifos and parathion are commonly used organophosphate insecticides in agricultural, household and industrial/commercial settings. In a biological system, each of these pesticides is activated by phase I (P-450) reactions to their respective active forms, chlorpyrifos-oxon and paraoxon. These metabolites are potent inhibitors of the neuronal acetylcholinesterase. Thus, interactions between these pesticides can occur at two levels in the organism: a) the P-450 enzymatic bioactivation site, and b) acetylcholinesterase binding site. The objective of this study was to develop a physiologically based pharmacokinetic (PBPK) model to predict the joint toxicity of these chemicals in the rat. Model parameters were either calculated, obtained from published animal studies, or optimized using available data. PBPK models were developed for each chemical and its respective metabolite. The metabolite concentrations in blood were then linked to an additional model describing the kinetics of acetylcholinesterase inhibition and aging. The developed overall model was used to investigate the interaction between the chemicals with their respective biological sites. A response surface was constructed by using the overall model to simulate the response (percent of inhibited acetylcholinesterase) as a function of dose of each single chemical or various binary mixture combinations. For instance, using an oral dose of 3mg/kg of each chemical, the model predicted maximum acetylcholinesterase inhibition at 35% and 20% of its initial level by parathion and chlorpyrifos, respectively. Although parathion produced a larger maximum inhibition, chlorpyrifos produced a longer acetylcholinesterase inhibition profile. This study illustrates the use of models to analyze toxicological interactions at dose levels that cannot be tested experimentally.

SARIN-INDUCED NEUROTOXICITY AND INCREASE IN BLOOD BRAIN BARRIER (BBB) PERMEABILITY ARE AUGMENTED BY STRESS AND COMBINED EXPOSURE TO PYRIDOSTIGMINE BROMIDE (PB), DEET, AND PERMETHRIN IN RATS.
E. M. Elmasy, A. A. Abdul-Rahman, A. K. Sherry and M. B. About-Dania. Pharmacology and Cancer Biology, and Surgery, Duke University Medical Center and VA Medical Center, Durham, NC.

We studied the effect of 28 daily chemical exposures to PB (1.5 mg/kg, po. in water), DEET (40 mg/kg, d, in ethanol) and permethrin (0.13 mg/kg, d, in ethanol) and stress by placing the animals in a Flexiglas restrainer for 5 min daily for 28 days on sarin (single 50 microgram/kg, 9.5 LD50, dose, im, in saline)-induced toxicity. A total of seven groups of rats were used for the treatment regimens: chemicals, stress, chemicals+stress, sarin, sarin+chemicals, sarin+chemicals+stress, and vehicle control. Brain acetylcholinesterase was inhibited in the cortex, midbrain and brainstem with sarin+chemicals+stress causing most inhibition. Only sarin+chemicals+stress led to significant inhibition of plasma brain cholinesterase. Chemicals and sarin chemicals increased ligand binding to the muscarinic acetylcholine receptor. Treatment with sarin+chemicals+stress increased uptake of [3H]hexamethonium iodide by brain regions in the descending order midbrain > brainstem > cerebellum > cortex. Increased permeability of BBB was confirmed using anti-EBNA monoclonal antibodies and horseradish peroxidase. Histopathological studies with H&E stained showed much greater density of dying (degenerating) neurons in animals treated with sarin+chemicals+stress than other treatments in the cerebral cortex, hippocampus, and cerebellum, where Purkinje cells were more sensitive than molecular cells. Animals treated with sarin+chemicals+stress exhibited greatly enhanced gliarial fibrous scar protein staining, characterized by hypertrophy of astrocytic processes, that correlated with cell death recognizable with H&E stain. These results demonstrate that neurotoxicity caused by a single sarin exposure is enhanced by combined exposure to stress, alone or with PB, DEET, and permethrin at real-life doses. Supported in part by the US Army Medical Research and Material command under contract DAMD 17-98-0-C-0027.

174 URINARY EXCRETION KINETICS OF TRICHLORACETIC ACID (TCA) AND DICHLORACETIC ACID (DCA) IN RATS EXPOSED TO A CHLORINATION-BY-PRODUCT (CBP) MIXTURE IN DRINKING WATER.
G. Balogopul1, F. Wui2, K. Froese3, S. Hrudey2 and K. Krishnan1. 1-Groupe de recherche en toxicologie humaine (TOXHUM), Université de Montréal, Montréal, PQ, Canada and 2-Dpt of Public Health Sciences, University of Alberta, Edmonton, AB, Canada.

Non-invasive biomarkers such as urinary concentrations of parent chemicals and metabolites are potentially useful for exposure assessment. Haloacetic acids, such as TCA and DCA, are potential biomarkers of exposure to CBPs in treated drinking water. However, there has not been an experimental evaluation of their urinary excretion kinetics during controlled exposure to CBP mixtures. The objective of the present study was therefore to characterize the urinary excretion kinetics of TCA and DCA in animals exposed to CBP mixtures. Male Sprague-Dawley rats (180-200 g) were provided ad libitum drinking water fortified with a mixture of CBPs (TCA, DCA, monochloroacetic acid, chloroform, bromoform, bromodichloromethane and dibromochloromethane) at two exposure levels for 21 days. The low exposure group received drinking water in which the CBP concentrations were similar to the highest concentrations reported in a Canadian drinking water survey, whereas the high exposure group received water fortified with ten times the highest reported environmental concentration of each CBP. Daily body weight of the rats, water consumed and the urinary output were quantified. The daily, composite urine samples from individual animals were analysed for TCA and DCA using a gas chromatograph with an electron capture detector. For the low
dose group, the daily urinary excretion of TCA varied from 0.043-0.05 mg/L and that of DCA ranged between 0.001-0.025 mg/L, whereas these ranges were 0.84-4.1 mg/L and 0.025-0.25 mg/L, respectively, for TCA and DCA in the high exposure groups. The average % dose eliminated in urine during the experimental period was 60 for TCA and 2.2 for DCA, for the low dose exposed. This controlled exposure study conducted with the rodents suggests that urinary TCA may be developed as a useful biomarker of low-level exposure to CBP mixtures.

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

K. Krenz and M. Beigoune. Groupe de recherche vs toxicologie humaine (TOMHUM), Universite de Montreal, Montreal, PQ, Canada.

The objective of this study was to develop and validate a binary interaction-based algorithm for predicting the blood concentrations of VOCs in mixtures. The approach involved the calculation of the blood concentration at steady-state of one chemical (Cd) in the presence of other chemicals competing for hepatic metabolism. The following algorithm was developed: Cd = Cs-ind (1 + Kinh (Cd)(Kinh(Cd))) where Cs = concentration of the substance in the mixture, Cs-ind = concentration of substrate when present individually, Kinh = concentration of the inhibitors in the mixture, Am = maximal augmentation of Cs possible in the mixture, and K = inhibition constant specific for each inhibitor-substrate combination. Cs-ind was estimated from steady-state algorithms derived from physiologically-based pharmacokinetic (PBPK) models. Both Am and K were derived from mechanistic studies with each chemical mixture. Knowing the Cd measured during mixed exposures, the Cs was calculated on the basis of information on binary interaction mechanisms. The above algorithm was validated using the hepatic venous blood concentration (Cv) of benzene (B) found in mixtures along with dichloromethane (D), toluene (T), ethylbenzene (E), and m-xylene (X). The Cd corresponding to D, T, E, and X in mixtures were obtained with a previously validated physiologically-based pharmacokinetic (PBPK) model, and were used to predict the modulation of Cv for B during mixed exposures. The computed Cv (µg/L) of B was 1.95 for single chemical exposure whereas it was predicted to increase to 3.97, 13.1, 26.7, 35.5, respectively, in the presence of D, T, E, and D + T + E, respectively. These values are identical to those obtained using the validated mixture PBPK model. The results presented in this study demonstrate the possibility of predicting the blood concentrations of chemicals during mixed exposures from knowledge of binary chemical interactions.

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HEPATOTOXIC EVALUATION OF THE BINARY INTERACTIONS OF CHLOROFORM (CHCl3) WITH BROMODICHLOROMETHANE (BDCM), CHLORODICHLOROMETHANE (CDCM) AND BROMOFORM (CHBr3).

Y. M. S. Sev, C. Georgias, A. McDonald, L. K. Teuchscher and J. E. Simpson. NHUER/EROD, Research Triangle Park, NC, USA, 4 EVV/chemical, and NCEA/EROD, Cincinnati, OH.

Trichloroethanes (TMs) result from chlorination or chloramination of natural organic matter in water. Acute and chronic exposure to individual TMs results in target organ toxicity (hepatic, renal) and carcinogenicity. Since human exposure is to multiple chemicals, toxicity data on mixtures of THMs are needed. There are three experiments conducted to examine the binary interactions of CHCl3 with the other 3 THMs. Each experiment consisted of an aqueous control group: CHCl3, alone at 0.1, 1.0 or 3.0 mg/ml/day; or BDCM alone, CDM alone or CHBr3 alone at 0.1, 1.0 or 3.0 mg/ml/day; and 3 mixture groups of the corresponding CHCl3, TCMs mixture at a 1:1 mixing ratio and total dosages of 0.1, 1.0 or 3.0 mg/ml/day; and 2 mixture groups at total dosages of 1.0 or 3.0 mg/ml/day and varied mixing ratios (2.5:1: CHCl3; BDCM; 6.5:1: CHCl3; CDM; 65:1: CHCl3; CHBr3) based on the average seasonal proportions of the THMs at 35 water treatment plants (Johnson et al., 1989). Female CD-1 mice were gavaged daily for 14 days. Hepatotoxicity was assessed by serum SDH. The experimental SDH means were compared with 95% prediction intervals generated by a threshold additivity model built under an assumption of dose addition (Gennings et al., 1997). For CHCl3; BDCM, the closest of the predicted response under an additivity model and observed mean values indicated no deviation from dose additivity.

For CHCl3; CDM, deviation from dose additivity was not detected for any dose level at either mixing ratio. For CHCl3; CHBr3, significant departure (antagonism) from dose additivity was detected only at 3.0 mg/ml/kg of the 1:1 mixture; this result must be viewed with caution as the sample size was small in this group due to mortality. In summary, the present data indicate that the binary interactions among the THMs at these mixture dosages and mixing ratios are either additive or antagonistic, but not synergistic. This abstract may not reflect EPA policy.

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MICROBIAL GENOTOXICITY OF MODEL CHLORINATED ALIPHATIC HYDROCARBONS AND BINARY MIXTURES.

K. C. Donnelly, T. C. Hudson and M. M. Murtaz. Veterinary Anatomy & Public Health, Texas A&M University, College Station, TX, Environmental & Occupational Health, Texas A&M University, College Station, TX and Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Human exposure typically occurs to complex chemical mixtures. This includes environmental exposures, as well as the various mixtures of chemicals in our food. This study addresses the development of an in vitro methods for assessing the toxicity of complex chemical environmental mixtures, and is focused on binary mixtures of chlorinated aliphatic hydrocarbons, Tetrachloroethylene (TCE) is one of the most commonly used trichloroethylene (TCE) is one of the most common groundwater contaminants found near hazardous waste sites. The genotoxicity of these model chemicals and binary mixtures of chemicals was measured in the Salmonella/microsome mutagenicity assay. Chemicals were tested both with and without metabolic activation. TCE and dichloroethene (DCE) were tested as liquids by adding various concentrations of the chemicals in glass petri dishes and putting the dishes in cellophane bags with agar plates containing the microorganisms. V was tested as a gas by filling the cellophane bags containing the agar plates with a predetermined volume of gas ranging from 0 - 50% V. Neither TCE nor DCE were mutagenic with activation. Vinyl chloride (VC) induced a doubling of revertants at all doses tested, with the maximum response of 3.46 for TA100 revertants observed as dose of 50% VC. There were no appreciable differences in the response induced by VC alone and the response induced by binary mixtures of TCE or VC. The mixture of 30% VC and TCE induced 325 net TA100 revertants. Data from the microbial mutagenicity assay indicate that additive responses were induced by mixtures of VC with non-genotoxic chlorinated aliphatic hydrocarbons.

Supported by NIEHS SBIR Grant No. 2P2 ES05491 and ATSDR Grant No. TUB8450502.

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

J. Sweneyowa, B. Yilmaz, D. O. Carpenter and K. F. Assoc. School of Public Health, University at Albany, Rensselaer, NY and Environmental Science, University of Massachusetts, Amherst, MA.

Fourteen individual polychlorinated biphenyls (PCBs) were identified for the estrogenic and antiestrogenic activity in human breast cancer cell lines. The reconstructed mixture contained IUAPC Nos.: 28, 77, 101, 105, 118, 126, 138, 146, 153, 156, 169, 170, 180, 187, and was prepared to reflect the pattern and concentration of PCBs frequently detected in human breast milk. The assay for estrogenic/antiestrogenic activity, the MCF-7 focus assay, is based on postconfluent cell growth and tissue restructuring measured as the development of multicellular layers or foci. The individual PCBs and the reconstructed mixture were tested at 5 concentrations between 1.6 x 10^5 M and 5 x 10^4 M. Two of the 14 PCBs (Nos.: 101 and 138) were estrogenic; they induced the formation of foci at the highest test concentration. Five of the 14 PCBs (Nos.: 28, 77, 125, 125, and 169) were antiestrogenic; they inhibited the foci induced by 1 x 10^5 M mirex. The reconstructed mixture was antiestrogenic at the three highest test concentrations. The results are compared and discussed in relation to a published report (Han et al., 1999, TAP 128, 231-243) in which treatment of female rats with the reconstructed mixture resulted in increased uterine wet weights of their offspring. (Supported by NIH ES067975-01A1 and TUBITAK-NATO)

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


Individual petroleum products are complex mixtures consisting of hundreds of individual components. The assessment of human health risk is complicated by the lack of toxicity data for the exact mixture of components, although data are available on related components. Recently, a day oral gavage toxicity study was conducted in rats and mice for the equivalent carbon (EC) aromatic fraction (AF) of jet fuel, containing some aliphatics (Smith et al., 2000). Toxicol. Sciences. 54(1):356). Using this study, we are developing a reference dose (RFD) for the AF. In order to determine if uncertainty factor (UF) values derived from mechanistic studies can be used to account for subchronic to chronic extrapolation and uncertainty due to an incomplete database, we investigated which individual compounds and mixtures are relevant to the toxicological evaluation of this fraction and could support the derivation of data-derived UFs. A database was constructed using dose-response data.
from published studies of chemicals within the AF including styrene, ethylenebenzene, xylene, cumene, 1, 3, 5-, 1, 2, 4- and 1, 2, 3-trimethylethylene, high flash aromatic naphtha (HFAN), naphthene, 1- and 2-methylethylbenzene, butadiene and AF. Dose-response data were adjusted to common dose and response metrics for comparison across endpoints grouped by target organ. Evaluation of the database suggests that components of the fraction can provide insight into the expected toxicological behavior of the fraction (e.g., dose-response relationships were similar for rats across hematological, liver and neurotoxicity endpoints for AF, biphenyl, HFAN and camene). The availability of data for the AF mixture and for some of its components provides an opportunity to evaluate approaches for assessing the toxicity of petroleum-derived mixtures.

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

C. J. Bogert, J. T. Gross, P. D. Gning, T. G. Osmont, B. Price

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

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

E. F. Madden, B. A. Fowler and M. Akkerman

Arsenic and cadmium are toxic to renal proximal tubule cells but the single and combined effects of these elements on regulatory processes such as protein phosphorylation, stress protein response, and the caspase system have not been fully studied. The present studies examine the effects of As and Cd alone or in combination over concentrations of 10^-10 to 10^-7 M in the rat NRK-52E and human HK-2 kidney cell lines. Preliminary TUNEL assays under these exposure conditions have shown only a minority of cells die from apoptosis. Hence, western immunoblots assays were conducted using antibodies to phosphorylated tyrosine, caspase-3, heme oxygenase, HSP 25, and HSP 27. Results showed marked increases in tyrosine phosphorylation for single and combined elements at 10^-7 concentrations in both cell lines. Increased heme oxygenase levels were observed to either coincide with increased tyrosine phosphorylation and/or to precede it at lower doses of 10^-9 or 10^-8 M. Decreased levels of caspase-3 were generally found to coincide with increased tyrosine phosphorylation at 10^-7 concentrations of single and combined elements and at 10^-7 As x Cd. In HK-2 cells this was accompanied by decreased levels in HSP 27. In rat NRK-52E cells decreased levels in HSP 25 were observed only for 10^-7 M of As x Cd. DNA fragmentation assays of NRK-52E cells showed no measurable positive results for apoptosis. Western immunoblot analysis of protein kinase C and tyrosine phosphatase PAC-1 in HK-2 cells showed decreased levels for 10^-7 M doses of single and combined elements. These studies suggest single and combined mixtures of As and Cd may act in concert to inhibit essential regulatory processes leading to apoptosis and thus lead to cell death primarily via necrosis. Oxidative stress may play a role as evidenced by elevated heme oxygenase levels and increased levels in phosphorylated tyrosines found for higher doses of single and combined elements may be due to disruption of metabolic oxidative phosphorylation. (Supported by EPA STAR Grant #227161 and ES 7299-06).

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

D. K. Craig, Rusk Technology, Westminster Safety Management Solutions LLC, Aiken, SC.

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

183 ALTERNATIVE 5'-SPlicing of HUMAN MICROSMAL EPoxide HYDROLASE: GENETIC LOCATION and CHARACTERIZATION OF VARIANT EXONS.

C. Hassel, A. Gao and C. J. O'omiecienski.

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

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


University of Michigan Medical School, Ann Arbor, MI and Pediatrics, University of Michigan Medical School, Ann Arbor, MI.

Hsp27 is believed to stabilize actin filaments, but the mechanism is unknown. We identified hic-5, a focal adhesion protein and androgen receptor co-activator (ARASS), as an hsp27 binding protein, and have mapped the interaction to the hic-
5 C-terminal LM4 domain and the hsp27 C-terminal domain. We observed that hsc-5 inhibits the thermo-protection provided by hsp27. Hsp27 has been suggested to protect cells from heat by stabilizing actin filaments and interfering with hsp27-mediated actin filament stabilization. We analyzed the effect of hsp27-5 interaction on cell polymerized actin (F-actin) using phallolidin binding assay in COS cells transiently transfected with vector only or hsp27, hsc-5, hsp27 + hsc-5, or hsp27 + hsc-5 SALM4 (hsc-5 mutant that does not bind hsp27) constructs. Hsp27 transfected had more and higher hsc-5 transfected had less cell F-actin compared to vector transfected cells. However, expression of hsc-5, but not hsc-SALM4, with hsp27 reduced cell F-actin content. Cell fractionation studies in identically transfected NIH 3T3 cells lysed in buffer containing 0.1% Triton X-100 revealed hsc-5 in the soluble fraction in hsc-5 transfected, but redistribution of hsc-5 to the Triton-insoluble fraction (that also contains actin) was blocked by hsp27 cotransfection. Immuno-staining of murine podocytes stably transfected with vector control, hsp27, or hsc-5 demonstrated reduced actin stress fibers and focal adhesions in hsc-5 transfected compared to vector or hsp27 transfected cells. We conclude that hsc-5 inhibits hsp27-mediated actin filament stabilization, hsc-5 may interfere with the formation of focal adhesions, and hsp27 can regulate the intracellular distribution of hsc-5. Supported by NIH grants R01-ES06265, R01-ES07006 and P01-ES1188 to MJW, and K08-DK02455, R01-DR55602 and a research grant from the National Kidney Foundation of Michigan to WES.

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


DNA double-strand breaks (DSB) in yeast are repaired primarily by homologous recombination (HR) involving several proteins including Rad54 and Rad54q which interact during heteroduplex DNA formation. Rad54q alters DNA double helix formation, in an ATP-dependent manner. We examined DSB-induced cell killing, HR, and chromosome loss in a/a and a/a diploid yeast with normal Rad54 levels, overexpressed wild-type Rad54, or overexpressed mutant versions of Rad54 defective in ATP binding or ATP hydrolysis. DSBs were introduced into a/a and by HO nuclease. The Heber Lab. showed that excess wild-type Rad54 reduced methyl methane sulfonate (MMS) resistance by 25%, while excess ATP binding or hydrolysis T mutations Rad54 reduced MMS resistance by 39%. In contrast, DSB-dependent cell killing with excess wild-type or mutant Rad54 was minimal in a/a cells, and modest (25%) in a/a cells. MMS is an amplifying agent and its cytotoxic effects are probably replication-dependent, so the different effects of excess Rad54 with MMS and DSB damage may reflect differences in Rad54 function during replication (i.e., during lesion bypass at blocked replication forks by sister chromatid exchange) replication independent DSB repair. In a/a and a/a cells, excess wild-type Rad54 had no effect on frequencies of DSB-induced HR or chromosome loss, but excess mutant Rad54 reduced HR by 1.5- 3-fold. In all cases, reduced HR correlated with increased chromosome loss. We reasoned that excess wild-type Rad54 might enhance Rad51-mediated heteroduplex formation and increase gene conversion tracts length, but the opposite was observed; excess mutant Rad54 had no effect on tracts length. Together these results suggest that excess wild-type Rad54 does not affect HR initiation but negatively affects the later step of heteroduplex formation, whereas excess mutant Rad54 blocks HR initiation (leading to chromosome loss and some cell killing) but does not affect later steps.

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

K. E. Stine, G. E. White, J. P. Ellwitz, K. A. McFarland, R. A. Boorpan and N. D. Smith. Department of Biological Toxicology, Ashland University, Ashland, OH.

The classic heat shock or stress response has been studied in a number of organisms including the nematode C. elegans. We have previously demonstrated that elevated temperatures as well as exposure to the mitochondrial toxicant sodium azide induced the ability to induce a stress response in this nematode, and that this response involves induction of both hsp-16 and hsp16. Now, we have isolated substrates of the N2 wild type nematode which show intrinsic thermotolerance, defined as a significantly increased survival probability compared to controls when taken from either 16° or 25° C directly to 37° C for two hours. These N2-TiS have maintained their intrinsic thermotolerance for well over two years, and in addition, show a significantly increased life span. Experiments have shown that the average lifespan for worms from the N2-TiS strain grown at 25°C is 14.5 ± 0.7 days, compared to 12.5 ± 0.5 days for N2 controls. This represents a 16% increase in average lifespan for this strain. It was also noted that in every experiment the life span of at least 2-3 N2-TiS' worms was significantly longer than the mean (typically on the order of 19- 20 days) in terms of stress protein expression. Western blot analysis of the N2-TiS strain indicates elevated hsp16 levels when compared to controls. To further characterize the IT state, we have designed primers and initiated RT-PCR studies from both N2TiS and N2 controls to determine hsp70, hsp60, and hsp90 expression in these worms. In less than two years we have maintained N2TiS substrains for over 2 years, and have discovered that these populations show significantly increased longevity compared to N2 controls. In addition, we have demonstrated that these IT substrains show elevated levels of at least one stress protein, hsp16, when compared to N2 controls. We are continuing to investigate the molecular mechanisms by which this IT state is maintained through application of RT-PCR techniques.

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SULFUR AMINO ACID DEPRIVATION INDUCES MICROsomAL EPoxide HYDROLASE, A PUTATIVE CELL SURFACE AUTOANTIGEN, IN PARALLEL WITH APOPTOTIC CELL DEATH.

K. W. Kang, Y. G. Kim,1 R. E. Nocki,1 and S. G. Kim.1 *National Research Laboratory (MDT), College of Pharmacy, Seoul National University, Seoul, South Korea and 1Institute of Environmental Health Sciences, Wayne State University, Detroit, MI.

Microsomal epoxide hydrolase (mEH), a detoxifying enzyme and putative cell surface autoantigen, is inducible by xenobiotics and by certain pathophysiological conditions (e.g., tumorigenesis and protein-calorie malnutrition). The present study was designed to determine mEH expression in H411E cells during apoptosis induced by sulfur amino acid deprivation (SAD) and to identify the signaling pathway for the enzyme induction. SAD induced apoptotic cell death at 48-72 h and decreased mitochondrial permeability with cytochrome c release, both of which were prevented by SB203580 or by dominant-negative JNK1 (JNK1(4) transfection). Either caspase-3 activity or poly(ADP-ribose) polymerase cleavage increased during SAD-induced apoptosis, indicating that SAD-induced apoptosis independent of caspase activation. This was supported by the observation that benzoxycarbonyl-Val-Ala-Asp-fluoromethyl ketone, a general caspase inhibitor, did not prevent apoptosis. The levels of mEH mRNA and protein were notably increased in cells under SAD for 48-72 h. The induction of mEH occurred in parallel with apoptotic cell death. Whereas SAD-induced apoptosis resulted from both JNK1 and p38 kinase activation, mEH induction was decreased only by JNK1(-) transfection. Immunocytochemistry revealed that mEH protein was intensely stained in apoptotic cells, cellular fragments and cell debris. The number of cells positive for surface mEH also substantially increased by SAD, as evidenced by flow cytometry analysis. These results demonstrated that SAD induced apoptosis with an increase in cytochrome c release from mitochondria, but not through caspase-3 activation, and that apoptosis induced by SAD accompanied mEH induction, which involves JNK1, but not ERK1/2 or p38 kinase pathway. The expression of cell surface mEH by SAD may be associated with presentation of autoantigen.

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COVALENT BINDING OF ACID IS REQUIRED FOR NUCLEAR TRANSLocation OF NF-E2-RELATED FACTOR 2 (NF-E2) AND ANTIOXIDANT RESPONSE ELEMENT (ARE) ACTIVATION.


Expression of phase II detoxifying genes is regulated by Nrf2-mediated activation of ARE. Phosphorylation stimulated 3-kinase is involved in the translocation of cytoplasmic Nrf2 into the nucleus through rearrangements of actin in response to tert-butyhydroquinone (t-BHQ). The apparent molecular weights of cytoplasmic and nuclear proteins cross-reacting with anti-Nrf2 antibody varies according to reducing condition. The present study was initiated to determine the apparent molecular weight of Nrf2 could covalently bind with actin. The apparent molecular weight of Nrf2 was 57 kDa in both cytoplasmic and nuclear proteins fractionated from cell lysates prepared in the presence of dihydrothiol, whereas in the absence of dihydrothiol, that of the nuclear proteins immunologically reacting with anti-Nrf2 antibody was 100 kDa. The band intensity of the nuclear 100 kDa protein was increased by t-BHQ treatment. Nuclear translocation of cytoplasmic Nrf2 by t-BHQ was prevented by pre-treatment of cells with dihydrothiol. Hence, it is highly likely that Nrf2 translocates into the nucleus as a complex covalently bound with a 43 kDa protein.
Western blot analysis revealed that anti-actin antibody recognized a 100 kDa nuclear protein as well as 43 kDa actin protein. The level of the 100 kDa nuclear protein, which was immunoprecipitated with anti-NF-κB and immunoreactivity with anti-actin antibody, increased in cells treated with t-BHQ. Also, the ARE band intensity was reduced in gel-shift analysis after immunoprecipitation of the nuclear extract with anti-actin or anti-NF-κB antibody. Addition of dithiothreitol and beta-mercaptoethanol to the nuclear fraction failed to convert the 100 kDa protein to 70 kDa NF-κB. Hence, it is unlikely that NF-κB binds with actin through disulfide bonding. These results demonstrated that the altered cellular redox-state induced by oxidative stress causes NF-κB to bind with actin and the actin-bound NF-κB translocates into the nucleus prior to its ARE activation.

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

S. K. Kneuzeug, S. R. Martin, L. L. Rube, and D. E. Williams. Environmental & Molecular Toxicology & The Linus Pauling Institute, Oregon State University, Corvallis, OR and La Jolla Institute for Experimental Medicine, San Diego, CA.

The most common allele for the major mammalian lung isoenzyme of flavin-containing monooxygenase, FMO2, encodes truncated inactive protein due to a C to T transition that results in a premature stop codon. The allele (fmo2*2) encoding full-length protein is absent in all Caucasian and Korean individuals genotyped to date; however, it is present in 26% of African-Americans. We recently demonstrated activity of this enzyme in lung microsomes from a heterozygous organ donor. FMO metabolism of the oxygenate of many nitrogen and sulfur containing drugs/xenobiotics. The present study focused on extraction of ethnic genotyping to determine the prevalence of fmo2*2 among Hispanics, and assessed the activity of candidate substrates with baculovirus expressed fmo2*2, to assess the potential relevance of allelic variation. Genomic DNA was isolated from lung, liver or blood (plasma or lymphocytes). Allele-specific forward primers, differing in length by 15 nucleotides and one common reverse primer were designed to produce a 245 bp PCR product corresponding to the allele (fmo2*2) encoding truncated protein, and a 230-bp product corresponding to fmo2*2. Allele-specific PCR products were identified by PAGE. DNA from 21 individuals was sequenced including samples putatively containing the fmo2*2 allele, to obtain genotypic verification. To date 40 Hispanic individuals have been genotyped; 4.5% have the fmo2*2 allele. Baculovirus expressed fmo2*2 was active toward substituted thiourea compounds with a small cross-sectional area (4.3 Å), but was inactive toward 1,3-diphenylthiourea (11.2 Å), in assays following NADPH oxidation. Thioacetamidemia and thioacetamide were also good substrates. The presence of the ethnic fmo2*2 allele in African American and Hispanic populations indicates the potential for ethnic differences in drug and environmental toxicant metabolism. (Supported by PHS grant HL07900.)

APPLICATION OF THE RNA INTERFERENCE ASSAY TO INVESTIGATE THE ROLE OF SP1 AS A TRANSCRIPTION FACTOR IN BREAST CANCER CELLS.

M. H. Abdelrahim, I. Samudio and S. H. Safa. Veterinary Physiology & Pharmacology Texas AM University, College Station, TX.

Sp1 is a member of the Sp family of transcription factors that play an important role in regulating basal expression of multiple genes through interaction with GC-box-rich promoter sequences. The estrogen receptor (ER) interacts with Sp1 protein, and several estrogen (E2)-responsive genes are induced in human breast cancer cells through ligand-dependent activation of ERα/Sp1. This study investigates RNA interference (RNAi) through transcriptional and/or posttranscriptional gene silencing of Sp1 protein in MCF-7 cells as a probe for confirming the role of Sp1 and ERα/Sp1-mediated transcription. For the proposed studies in subconfluent MCF-7 human breast cancer cells, we have used a double-stranded 21 nucleotide small interfering RNA (siRNA) that is homologous in sequence to the coding region 1011-1032 relative to the start codon of Sp1. Lipofection was used for transfection of 0.75 ng siRNA per well. Cells were then harvested after 48 hr and Sp1 protein was examined in whole cell extracts by Western blot analysis. The results showed that immunoreactive Sp1 protein levels were decreased by 60 - 70%. In parallel studies, MCF-7 cells were transfected with a construct (pSP1) containing a single consensus GC-rich Sp1 binding motif, and reporter gene activity was determined in the presence or absence of cotransfected siRNA. The results showed that siRNA decreased Sp1-dependent transcription by > 90%, whereas in control experiments using siRNA for lamin B, no effects on Sp1 protein levels or p50-dependent activity were observed. The RNAi assay is currently being used to target other Sp1 and ERα/Sp1-dependent genes/promoters to investigate contributions of Sp1 and other Sp1 proteins as transcription factors. (Supported by NIH CA76656 and ES09105)

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

K. Tokio, S. S. Lai and J. L. Marks. Center for Molecular and Cellular Toxicology, Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas at Austin, Austin, TX.

The rapid formation of DNA single strand breaks, increases in histone H3 phosphorylation, and the dephosphorylation of histone H2A, precede reactive oxygen species (ROS) induced oncotic cell death in renal proximal tubule epithelial cells (LLC-PK1) [Mol Pharmacol, 60:394-402, 2001]. Consistent with these findings, we now report that ROS, generated following the addition of 2, 3, 5-tris(4-iodoato)phenyl-4-hydroquinone to LLC-PK1 cells, causes the rapid ribonucleation of histones H1>H3 and H4. Histone ribonucleation precedes histone H3 phosphorylation, suggesting that ribonucleation of linker H1 in particular, provides subsequent access for the requisite kinases to core histone H3. Moreover, ROS induced histone phosphorylation appears to be restricted to a small subset of nucleosomes that are subject to hyperacetylation/ deacetylation. Thus, triton-oxecdased polyacrylamide gel electrophoresis analysis of nuclear extracts from LLC-PK1 cells treated with the histone deacetylase inhibitor, sodium butyrate, reveals that phosphorylated histone H1 co-migrates with hyper- (tetra) acetylated histone H3. The data suggest that putative histone H3 kinases selectively target nucleosomes that are also targets of butyrate sensitive histone deacetylation(s). The kinetics of the phosphorylation/acetylation coupling remains to be determined. Finally, in this model of ROS-induced oncotic cell death we fail to observe any changes in histone methylation. Because methylation of K9 and K27 in histone H3 are suggested to hinder K10 and N2H phosphorylation, the obtained data indicate that specific and precise histone modifications occur during ROS-induced cell death and that such selective histone modifications are supportive of the "Histone Code" hypothesis. (ES 07247, ES 07784)

TETRAFLUOROETHYLSTEINE-INDUCED ALTERATIONS TO MITOCHONDRIAL COMPLEX III ACTIVITIES IN VITRO.


The potent nephrotoxic tetrafluoroethylsteine (TFECE) induces cell death by initiating damage exclusively within the mitochondrial compartment. Nonetheless, de novo transcription of nuclear genes is a well recognized response to TFECE treatment in vivo and in vitro. Consequently, we examined the participation of communiation from mitochondria to the nucleus, analogous to a well-characterized communication pathway in Saccharomyces cerevisiae (retrograde regulation). RTC. Degenerative K1/PCR of structural motifs of the yeast RTC3 gene reproducibly yielded a 47-bp fragment identical to a segment of the mitochondrial cytochrome b component of Complex III (ubiquinol:ferrochypochrome oxidoreductase). Alterations to antimycin A-sensitive Complex III activities were examined in an accurate mammalian in vitro model for TFECE-induced cell death to determine the biological significance of this finding. TFECE treatment resulted in specific time-dependent decreases in Complex III activity (49.7 ± 0.8 mmol cytochrome c reduced/min/mg protein at 12 h vs 79.2 ± 1.2 mmol/min/mg protein control: mean ± s.d.; n=3, p<0.001). Cell viabilities were also determined by live/dead® and morphological changes by light microscopy. To determine if these changes correlated with a particular mode of cell death, antimycin A-sensitive Complex III activities were also evaluated after treatment with actinomycin D (DNA neosilus factor α, actinomycin, or other congeners. These results show a specific effect on Complex III activity by TFECE and suggest a mechanism by which changes to oxidative phosphorylation may be coupled to nuclear transcription via alterations to cytochrome b. (Supported by NIH grant GM52518 to SDN, GM51916 to SB and P50 ES70073 to the UW Center for Genecogenetics and Environmental Health.)

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

R. G. Conratty, K. W. Wilkie, S. F. Li, T. Chiang and C. M. Fenner. Department of Pharmacology, University of Miami, Miami, FL, National Cancer Institute, Bethesda, MD, Tufts University, New Orleans, LA and Department of Biology, West Virginia University, Morgantown, WV.

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

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

A. Nicolli, A. Moreto and M. Lotti. Dipartimento di Medicina Ambientale e Sanità Pubblica, Università di Padova, Padova, Italy.

Promotion of axonopathies is caused by certain esterase inhibitors such as sulfonyl halides, carbamates, thio-carbamates and phosphonates. Promotion was found to correlate with inhibition of a phenyl valerate esterase (PVE) activity in the whole nerve homogenate (M200). M200 was defined as the activity resistant to 40 μM paraxon and with an IC50 for mipafox of about 200 μM. Separated S-300 chromatography of the soluble portion of peripheral nerve separated most PVE activity (PVE-I) in fractions corresponding to 50-70 kDa MW proteins. P-SV was found to be selectively inhibited by promoters after in vivo treatment with several esterase inhibitors. At least 3 proteins, as seen from SDS-PAGE, were present in such fractions. It has been reported that P-SV activities are reversibly inhibited by paraxon (Barril and Villanueva, 1978) and the P-SV activity was inhibited by paraxon (2 μM, 20 min, 37°C, pH 8.0), then a 78% reversibility was observed after 24 hours dialysis at 0°C. Consequently, the P-SV IC50s for paraxon were 50 higher in the presence of paraxon. P-SV activity found to be sensitive to mipafox was about 90% of total, whereas that sensitive to tested inhibitors varied from 70 to 90%. The IC50s for mipafox were 20, 50 and >100 μM in the presence of 1, 8 and 40 μM paraxon, respectively, and 1 μM in the absence. Similarly, the P-SV IC50s for some promoters were found to be lower than those of M200. These were: for PMSF 1 and 60 μM, for L-tetrahydrodiphenylmethane 200 μM and 20 μM, for D-tetrahydrodiphenylmethane 400 μM and 5 μM, for 10-trans-stilbene 100 μM, and for miconazole 0.01 μM, respectively. In conclusion, P-SV activity displays in vitro sensitivity to promoters higher than that of M200. This is due to different assays which are required for either M200 or P-SV, because of the irreversible inhibition of P-SV caused by paraxon.

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

B. B. Aggarwal1, R. J. Anto1, A. Mukhopadhyay and C. G. Gaitonde. 'University of Texas M.D. Anderson Cancer Center, Houston, TX' and 'University of Kentucky, Lexington, KY.'

Cigarette smoking is a major risk factor for lung cancer and coronary artery disease. Pro-oxidant constituents of tobacco smoke have been implicated in smoking-associated disease development because of their potential role in inducing oxidative stress. We postulated that activation of redox responsive transcription factor, NF-KB, may play a role in tobacco smoke-induced cell damage. To test this hypothesis, activation of NF-KB was examined in cigarette smoke condensate (CSC)-treated human monocytic cells (U937). The cells were treated with CSC in serum free medium for 30 min. Nuclear and cytoplasmic extracts were prepared and analyzed for NF-KB binding by EMSA and IKBcointactness by immunoblots, respectively. Parallel experiments were carried out with TNF-α as positive control. The results showed that treatment of cells with CSC caused increased NF-KB DNA binding in a dose and time dependent fashion. Analysis of the cytoplasmic extracts showed that IKBα levels decreases in 15 min of CSC treatment of cells and were significantly diminished by 60 mins. Further experiments showed that IKBαinactivation was activated in cells with in 15 min of treatment with CSC. These observations indicated a potential of CSC to initiate phosphorylation and degradation of IKBα. Tests with nuclear extracts of various other CSC-treated cells of lymphoid and epithelial origin also showed increased NF-KB DNA binding suggesting that CSC-mediated NF-KB activation is in a cell specific. It is concluded that NF-KB activation by CSC may mediate tobacco smoke-induced cell damage (supported by NIH (P01 CA91844); Institutional Tobacco Funds, and KTRB-S-41165).

DUAL FUNCTIONS OF YEAST DNA POLYMERASE ε IN DNA LESION BYPASS.

A. Guo, X. Wu, P. Rechkiobit, N. Gescinov and Z. Wang. Graduate Center for Toxicology, 'University of Kentucky, Lexington, KY.'

Saccharomyces cerevisiae DNA polymerase ε (Polε) is required for DNA lesion bypass during replication. To gain insights into the function of Polε in lesion bypass, we have performed in vitro biochemical analyses of this polymerase in response to several DNA lesions. Purified yeast Polε performed limited translation synthesis opposite a template (~)-trans-anti-BENZO(A)PYRENE-N-7-guanine adduct by preferentially incorporating G and less frequently C. Opposite an acetalaminonaphthol (AAF)-aducted guanine, purified yeast Polε, predominantly incorporated a G. This lesion, however, significantly inhibited subsequent extension DNA synthesis. Extension synthesis by yeast Polε was more efficient when A or C was opposite the AAF-guanine. These results support the following dual functions of Polε. First, Polε catalyzes nucleotide incorporation opposite (~)-trans-anti-BENZO(A)PYRENE-N-7-guanine with a limited efficiency. Second, more efficient bypass of these lesions may require nucleotide incorporation by other DNA polymerases followed by extension DNA synthesis by Polε.

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

E. A. Calton, Y. Li and J. T. Zelikoff. Dept. of Environmental Medicine, New York University School of Medicine, Tuxedo, NY.

BENZO(A)PYRENE (BaP) is a widespread environmental contaminant and known carcinogen. In addition to its cancer promoting ability, BaP also has profound effects upon mammalian immunocompetence. Previous studies conducted in this laboratory have utilized a small teleost fish species, Japanese medaka (Oryzias latipes), as a laboratory model for investigating the immunotoxic potential of BaP. Similar to that observed in rodents, immune responses of medaka were suppressed following IP injection of BaP at doses that failed to affect morbidity or host survival. Specifically, BaP dose-dependently reduced antibody-forming cell (AFC) numbers. Furthermore, in order to test whether hepatic CYPIA activity mediated the immunotoxicity produced by exposure to BaP alone. This results suggests that BaP metabolism into reactive metabolites may be required for suppression of immune function. Further studies in this laboratory have revealed that injection of BaP at the same concentrations that suppressed AFC number, induced expression of CYPIA within a distinct subpopulation of kidney (primary site of hematopoiesis / leukopoiesis in medaka) mononuclear cells. Isolation of mononuclear cells from the kidney following in vivo exposure to BaP resulted in immunopositive staining of CYPIA within a small percentage of both glass-adherent and non-adherent immune cell types; little CYPIA expression was seen in isolated cells from the control vehicle. Moreover, exposure of isolated kidney immune cells to BaP in vitro resulted in an induction of CYPIA expression and a concurrent decrease in AFC number following in vitro immunization. This later finding suggests that specific medaka immune cells are sensitive to the parent BaP compound. Since it is apparent that medaka immune cells possess inducible CYPIA, it is possible that BaP may directly alter the function of these cells in the production of immunotoxic BaP metabolites in situ. (DAMD 17-99-901 and 60-1-8109)

EFFECT OF RETINOIC ACID ON 2, 3, 7, 8-TRETRETROXOBENZ(D)DIOXIN (TCDD)-INDUCED MATRIX METALLOPROTEASE EXPRESSION IN HUMAN KERATINOCYTES.


Exposure to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in an increased incidence of skin cancers. TCDD-exposure results in activation of the AhR/Arnt signaling pathway, mediating changes in gene expression through binding to XREs (Xenobiotic Response Elements). The AhR/Arnt pathway also interacts and influences other signaling pathways, including the retinoic acid (RA) pathway. Retinoids are powerful regulators of cell growth and differentiation and are widely used in the prevention and treatment of a variety of cancers in humans. Preliminary data from our laboratory suggests that interaction of these pathways alters the expression of matrix metalloproteinases (MMPs), proteins involved in tumor invasion. MMPs degrade the protein components of the extracellular matrix and the basement membrane, which is required for tumor cell migration and invasion. Northern analysis demonstrates that TCDD exposure of normal human keratinocytes results in activation of MMP expression, and that co-treatment with RA further stimulates MMP expression. The mechanism of activation of MMP-1 expression by TCDD + RA may involve binding to AP-1 sites in...
the proximal promoter, or potential XREs in the distal part of the promoter. Mobility shift data demonstrates TCDD activation of binding to the AP-1 site and to the XREs following TCDD treatment. Binding to a consensus XRE is abolished following RA exposure, whereas binding by the AP-1 site appears unchanged. Future experiments will examine the role of these elements in TCDD-RA induction of MMP expression, and to determine the effect of TCDD-RA treatment on cellular migration and invasion.

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TCDD STIMULATES ANDROGEN-RECEPTOR SIGNALLING IN LNCAP CELLS.

S. J. Barnes and A. Puga, Environmental Health, University of Cincinnati, Cincinnati, OH.

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

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DECI BASIC HELIX-LOOP-HELIQ DIFFERENTIALLY REGULATES GENES ENCODING CARBOXYLTERASES AND CYTOCHROME P450 ENZYMES.

Y. Li, M. Xie and B. Yan, Biomedical Sciences, University of Rhode Island, Kingston, RI.

DEC/STRA/SHARPs represent a new and distinct class of basic helix-loop-helix (bHLH) transcription factors. Human DECI is abundantly present in several normal tissues including heart, brain, placenta, lung and liver. In order to determine whether DECI regulates genes highly expressed in liver, transcription and repression activity towards several liver gene reporters was determined in stable transfected cell lines expressing DECI or a mutant (lacking the DNA binding domain). The expression levels of DECI or its mutant were inducibly regulated by tetracycline. The reporters were constructed to include a promoter fragment (0.2 kb) from genes encoding drug-metabolizing enzymes such as carboxylesterase and cytochrome P450 (CYP) enzymes. The stable transfected lines were cultured in the presence or absence of tetracycline and then transiently transfected with a promoter reporter and the pl-RK-TK plasmid (transfection efficiency control). After a 24-hr incubation, the reporter activities were determined with a dual luciferase system. The activity from cells cultured in the presence of tetracycline was used to compare with the activity of cells cultured in the absence of this antibiotic. The ratio of two values was used as an indicator of DECI-mediated effect. Among ten reporters used, four reporters including CYP2C8, 9, 19 and 34A were markedly repressed by DECI (-50%). The repression was observed only in cell line expressing full-length DECI but not the mutant, suggesting that repression of these reporters requires DNA binding. These results suggest that DECI may function as a competitor to the basal transcription factors in controlling the expression of these genes. The data presented likely provide a novel mechanism regarding the suppression of liver specific gene expression.

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SELECTIVE MODIFICATION OF CY5186 IN RAT LIVER CARBONIC ANHYDRASE II BY ACRYLONITRILE IN VIVO.

D. E. Netland, J. Cai and F. W. Benz, Pharmacology and Toxicology, Unit of Louisville Medical School, Louisville, KY.

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

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

H. Zhang and B. Yan, Biomedical Sciences, University of Rhode Island, Kingston, RI.

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

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

H. S. Youniss, A. R. Patriarch, J. J. Jiang and J. G. Sipes, Pharmacology & Toxicology, University of Arizona, Tucson, AZ and Medical Pharmacology & Toxicology, Texas A&M University, College Station, TX.

1, 2-Dichlorobenzene (1, 2-DCB), a potent hepatocytotoxic in Fischer-344 (F-344) rats, produces hepatocellular oxidative stress as a result of 1, 2-DCB bioactivation followed by a second oxidative burst mediated by activated Kupffer cells (KC). KC activation is responsible for the extensive hepatic necrosis associated with 1, 2-DCB. The hypothesis of this work is that KC activation, in part, is caused by cytokines and/or chemokines produced and released from compromised hepatocytes. Incubation of hepatocytes with 1, 2-DCB (3.6 umol) enhanced the production and release of cytokine induced neutrophil chemoattractant factor (CINC) by 2.2-fold and increased mRNA expression of macrophage migration inhibitory factor. These two proteins may activate KC. The production and release of CINC was not observed in hepatocytes incubated with 1, 4-dichlorobenzene (a nonhepatotoxic isomer) or 1-buthionine sulfoximine (to deplete glutathione). Interestingly, the receptor for CINC, CXCR Receptor 2, was expressed on KC as determined by immunochemical analysis. When conditioned media from 1, 2-DCB (3.6 umol) treated hepatocytes was incubated with KC these macrophages had increased transcription factor NF-kb binding activity (4.6-fold greater than control) and enhanced release of nitric oxide (21% above control). Collectively, these data suggest that 1, 2-DCB triggers a cascade of molecular processes in hepatocytes that promote the expression and release of oxidant sensitive chemokines. These products signal the activation of KC and the upregulation of the inflammatory response leading to the progression of 1, 2-DCB induced liver damage. (Supported in part by NIEHS Grant (ES06094), The Coca-Cola Foundation and an AFPE Fellowship).
204 DIORTHO CHLORINE SUBSTITUTED PCB CONGENERS ACTIVATE MITOGEN ACTIVATED PROTEIN KINASES INDEPENDENT OF PROTEIN KINASE ACTIVATION IN JB6 MOUSE EPIDERMAL CELLS.

B. V. Mediktar and O. Hernandez-Maldonado. Pediatric/ Human Development, Michigan State University, East Lansing, MI.

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants that evoke a variety of toxic effects including carcinogenicity in experimental models. The co-planar PCB congeners bind with the Ah receptor (AhR) and their toxic effects are most likely mediated through the AhR. The d-oriole chlorinated non-co-planar PCB congeners do not bind with the AhR. Their toxic effects may, therefore, occur independent of AhR. The mechanisms of their toxic interactions remain to be elucidated. In this study we examined the potential of two non-co-planar PCBs 2, 2', 4, 4'-tetrachlorobiphenyl (TCB) and 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl (HCB) to activate mitogen activated protein kinase (MAPK) cell signaling cascade in JB6 mouse epidermal cells. Activation of the extracellular signal regulated kinase (ERK) group of MAPKs in JB6 cells in response to PCB exposure was assessed by western blotting using phosphorylation specific site-specific antibodies. Treatment of the cells for one hour with 20 μM of either of these PCBs caused a six- to eight-fold increase in the activation state of the ERK-MAPKs. The activation of ERKs by these PCBs was also dose dependent and sustained for more than 12 hrs.

Pre-treatment of JB6 cells with a protein kinase C (PKC) inhibitor bisindolylmaleimide (5.0 μM) attenuated ERK activation by a potent PKC activator 12-tetradecanoylphorbol 13-acetate but not by the PCBs. These data suggest that ortho chlorinated PCBs may cause epigenetic alterations through activation of ERK-MAPKs independent of PKC activation (supported by NIHES Superfund grant no. ES04911).

205 THE AH RECEPTOR AND AH RECEPTOR NUCLEAR TRANSLLOCATION MEDIATE STIMULATION BY OLTIPRAZ OF THE XENOBIOIC RESPONSE ELEMENT IN THE RAT UGT1A1 GENE.

D. J. Auyeung, F. K. Keeler and J. K. Ritter. Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA.

The protective action of the chemo preventive drug, oltipraz (OTP), has been attributed in part to its selective inducing effects on phase 2 detoxifying enzymes such as glutathione-S-transferase (GST), NADPH quinone oxidoreductase (NQO), and UDP-glucuronosyltransferase (UGT) isozymes A6 and A17. The mechanism of induction of GST and NQO by OTP has been reported to be mediated transcriptionally via antioxidant response elements (ARE), but this has not been established for UGT1A6. Previously reported that OTP (50 μM) stimulated expression of a transfected UGT1A1 luciferase reporter plasmid in primary rat hepatocytes (PHH) and HepG2 human hepatoma cells. This effect required an intact xenobiotic-response element (XRE) located between bases -134 and -129. To confirm the role of the XRE in OTP inducibility, pGCL3-promoter constructs containing 1 to 5 copies of the UGT1A6 XRE conferred responsiveness to OTP in PRH. OTP induced CYP1A mRNA as well as transfected CYP1A reporter gene expression, indirectly supporting the ability of OTP to induce genes under XRE control. Two lines of direct evidence support the involvement of the arylhydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT) in mediating the effect of OTP on the UGT1A6 XRE. Co-transfection with AhR or ARNT expression plasmids enhanced UGT1A6 luciferase reporter plasmid induction by OTP. Electrophoretic mobility shift assays using UGT1A6 are highly probe and nucleic extracts from OTP and 3-methylcholanthrene treated HepG2 cells revealed that both agents induce specific complex formation on the UGT1A6 XRE. The specific binding was effectively competed by a CYP1A1 XRE oligonucleotide but not by a mutant UGT1A6 XRE oligonucleotide. Complexes were supershifted by antibodies to both AhR and ARNT. These results provide the first direct evidence that OTP may also induce biotransformation enzymes via an AhR-based mechanism and suggest that OTP transcriptionally activates both phase 1 and phase 2 enzymes. (Supported by ES07762 and ES078067).

206 NR2F-DEPENDENT NADPH:QUINONE OXIDOREDUCTASE EXPRESSION IN MOUSE CORTICAL ASTROCYTES.

J. Lee and J. A. Johnson. Molecular and Environmental Toxicology, UW-Madison, Madison, WI and School of Pharmacy, UW-Madison, Madison, WI.

The antioxidant responsive element (ARE) mediates transcriptional regulation of NADPH:quinone oxidoreductase (NQO1).Previous work in our laboratory showed that ARE activation depends on AhR and that oxidative stress is not involved in tert-butylnitroxide (tBNO)-mediated ARE activation in IMR-32 human neuroblastoma cells. In this study, we report that tBNO stimulation factor Nh2 plays a major role in transcriptional activation of NQO1 in mouse cortical astrocytes. For this study, we used primary cortical astrocytes of newborn pups from a Nh2+/Nh2- breeding. Nh2- astrocytes showed markedly decreased Nh2-1 enzyme activity and no induction by tBNO compared to Nh2+/or Nh2+/+ astrocytes. In addition, ARE-luciferase reporter gene expression was decreased in Nh2- astrocytes compared to Nh2+/+ or Nh2+/+ astrocytes in transient transfection experiment. Finally, decreased reporter gene expression was restored by overexpression of wild-type Nh2 in Nh2- astrocytes and ARE-luciferase reporter gene expression was completely blocked by overexpression of dominant negative Nh2. Previously we demonstrated Nh2 nuclear translocation is the critical event for ARE activation in IMR-32 cells. Similarly, tBNO treatment induced nuclear clearal of Nh2 in mouse cortical astrocytes. Taken together these data suggest that both basal and inducible NQO1 expression are dependent on Nh2 in mouse astrocytes. (Funded by ES08089, FS10047, BWF New Investigator Award).

207 INVOLVEMENT OF HEPATOCYTE NUCLEAR FACTOR-1 IN THE REGULATION OF RAT UDP-GLUCURONOSYLTRANSFERASE A16 (UGT1A16) GENE.

J. K. Ritter, F. K. Keeler and D. J. Auyeung. Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA.

UDP-glucuronosyltransferase A16 (UGT1A16) facilitates the removal of phenolic compounds from the body, thereby preventing their further metabolism and subsequent toxicity. UGT1A16 exhibits a complex pattern of tissue and inducer-specific expression. A possible basis for this complex pattern was suggested in a previous study identifying a second transcription unit (class 2) giving rise to UGT1A6. The class 2 transcript utilizes an alternative promoter designated "UGT1A16-2" which, on the basis of RTPCR and genomic sequence data, was found to be highly expressed in liver, kidney, and G2 tract relative to other extrahepatic tissues. The observation that an apparent hepatocyte nuclear factor-1 (HNF1)-binding site resides in the region immediately flanking UGT1A16P2 (bases -52 to -38) suggested a possible mechanism for this profile of expression. The current study was carried out to assess the functionality of this putative HNF1 binding site. DNAse I footprinting assays suggested specific binding by nuclear extract from rat liver to this site. This binding was confirmed using electrophoretic mobility shift assays. Nuclear extracts from rat liver and HepG2 cells exhibited high binding activity, whereas Hepa1 extract exhibited no detectable binding. The specificity of binding was confirmed in competition assays. Antibodies to HNF1β and to a lesser extent HNF1α resulted in a visible supershift in mobility of the complex. The functionality of this site in liver is supported by (1) the reduced expression of a UGT1A16P2 reporter in HepG2 cells when the HNF1 site is mutated and (2) the enhanced expression from UGT1A16P2 in both HepG2 and Hepa1 cells when either HNF1α or HNF1β are overexpressed. In contrast, the UGT1A16P2 promoter does not appear to contain a consensus HNF1 site and shows lower responsiveness to overexpression of HNF1α or HNF1β. These data provide a possible basis for the high expression of the UGT1A16 P2 promoter observed in liver and other tissues known to be enriched in HNF1. (Supported by ES07762 and ES078067).

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

J. P. Giesy1, W. Y. Hu2, P. D. Jones3, B. L. Upham2 and J. S. Troock1. 1National Food Safety and Toxicology, Michigan State University, East Lansing, MI and 2Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI.

Gap junctional Intercellular Communication (GJIC) is the major pathway of intercellular signal transduction, thus is very important for normal cell growth and function. Studies have demonstrated the ability of some perfluorinated organics to disrupt GJIC in in vitro cell culture assays. Recent studies have revealed a global distribution of perfluorinated compounds, especially perfluoroalkyl sulfonic acid (PFOS), in the environment. Therefore, the effects of PFOS, perfluoroalkyl sulfonate (PFAS), and perfluorobutylate sulfonate (PFBS) on GJIC were studied using rat liver epithelial (WB-F344) cell line and Carvan dolphin kidney epithelial (CDEK) cell line. Effects on GJIC were measured using the scrape loading dye technique. PFOS, PFOSA and PFBS were found to inhibit GJIC in a dose dependent fashion, and this inhibition occurred rapidly and reversibly. EC50s for inhibition of GJIC in rat cell lines were 29.96, 36.5 and 122 μM (15, 18.3 and 61 mg/L) for PFOS, PFOSA and PFBS respectively. PFBS showed no significant effects of GJIC within the concentration range tested. A structure activity relationship was established based on the four tested perfluorinated compounds, and it was indicated that the inhibitory effect was determined by the number of fluorinated tail carbon. GJIC function results from two cell lines were comparable to each other, it was also suggested that the inhibitory effects of selected perfluorinated compounds on GJIC were neither species- nor tissue-specific. The inhibition of GJIC observed in in vitro was confirmed in rats exposed to PFOS in situ with a LOEC based on liver accumulation of PFOS of 54 ppm.
(108 mg/L). Because of the relatively great concentrations of PFOS accumulated in liver in the in vivo experiments determination of NOECs based on tissue concentrations was not possible.

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DUSIFLAM INDUCTS SKELETAL MUSCLE DEGENERATION AND BEHAVIORAL ALTERATIONS IN LONG EVANS RATS.

K. J. Voigt-Blum and L. D. Trombetta. St. Johns University, Jamaica, NY.

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

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MERCURY INDUCES CYTOTOXICITY, AND TRANSRIPTIONALLY ACTIVATES STRESS GENES IN HUMAN LIVER CARCINOMA (HEPG2) CELLS.

P.B. Tchougouwou,1 D. Surton,1 N. Ninadvili and E. Shen.1 Molecular Toxicology Research Laboratory, Jackson State University, Jackson, MS and Xenomem Research Laboratory, Xenomem, Inc., Boulder, CO.

Mercury is a non-essential element that exhibits a high degree of toxicity to humans and animals. Exposure to mercury has been associated with a significant number of adverse health effects including: cardiovascular disease, anemia, developmental abnormalities, neurobehavioral disorders, kidney and liver damage, and cancer in some cases. In several studies, the toxicity of mercury has been attributed to its high affinity to protein-containing sulfhydryl groups. However, little is known regarding the molecular mechanisms by which mercury exerts its toxicity, mutagenesis, and carcinogenesis. This research was therefore designed to assess the cellular and molecular responses of human liver carcinoma cells following exposure to mercury. Cytotoxicity was evaluated using the MTT assay for cell viability, while the gene profile assay was performed to measure the transcriptional activation of stress genes in thirteen different recombinant cell lines generated from HepG2 cells. Cytotoxicity assay yielded a LD50 value of 3.5±0.6 μg/mL upon 48 hours of exposure, indicating that PCP is acutely toxic. A dose-response relationship was measured with respect to gene induction. For example, fold inductions of CYP1A1 were 1.0±0.0, 1.0±0.1, 1.3±0.5, 6.3±4.3, and 22.5±3.5 for 0.6, 12.5, 25, and 50 μg PCP/mL respectively. Overall, five out of the thirteen recombinant cell lines tested showed inductions to statistically significant levels (p<0.05). At 50 μg PCP/mL the average fold inductions were 22.5±3.5, 22±8.2, 8.4±1.9, and 12±3.6, for CYP1A1, XRE, HMTIII, c-fos, and GADD153, respectively. These results indicate the potential of PCP to undergo Phase I biotransformation in the liver (CYP1A1, XRE), to cause cell proliferation (c-fos), growth arrest and DNA damage (GADD153), and to influence the toxicokinetics of metal ions (HMTIII). Marginal inductions were recorded for HSP70, CRE, RARE, GADD45, and GADD51. Within the dose range 0.60 μg/mL tested, no significant inductions (p>0.05) were observed for GSTAT, NFκB, and p38RE. (Research supported by Title II Grant No. P031B990006).

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PHOSPHATIDYLINOSITOL 3-KINASE REGULATES ACTIVITY-RELATED NUCLEAR TRANSLATION OF NF-E2-RELATED FACTOR 2 (NF-E2) FOR RGST2A INDUCTION BY TEB7-BUTYLHYDROXYQUINONE.


Expression of phase II detoxifying genes is regulated by Nrf2-mediated antioxidant response element (ARE) activation. We previously showed that phosphatidyliso- tol 3-kinase (PI3-kinase) plays an essential role in ARE-mediated RGST2A induc- tion by oxidative stress. In view of the fact that the signaling pathways of platelet-derived growth factor (PDGF) β receptor tyrosine kinase (RTK) and PI3-kinase control microfilaments and transcription of actin-associated proteins, the current study was designed to investigate the role of these kinases in the rise in [Ca2+]i and actin-mediated transcription of cytoplasmic Nrf2 to nucleus. tert-Butylhydroquinone (t-BHQ) phosphorylated PDGF β receptor, and stimulated a rise in [Ca2+]i and nuclear translocation of Nrf2 in H4IIE cells, which were pretreated by pretreatment of the cells with PDGF-RTK or PI3-kinase inhibitors (AG1292; wortmannin / LY294002). Chelation of [Ca2+]i suppressed Nrf2 migration and t-RGST2A induction. t-BHQ relocalized Nrf2 to the cytoplasm with changes in actin microfilament architecture, as visualized by superposition of immunofluorescently stained Nrf2 and fluorescent phallolidin-stained actin. Furthermore, t-BHQ increased the level of nuclear actin, co-immunoprecipitated with Nrf2, which suggests that control of pretreatment of the cells with PI3-kinase inhibitors. Cytochalasin B, an actin disruptor, alone stimulated actin-mediated nuclear translocation of Nrf2 and ARE-dependent t-RGST2A induction. The ARE binding intensity was decreased by anti-Nrf2 or anti-actin antibody. In contrast, phallolidin, an agent that prevents actin filaments from depolymerization, inhibited Nrf2 translocation and t-RGST2A induction by t-BHQ. Thus, PI3-kinase signaling pathway regulates a rise in [Ca2+]i and rearrangement of actin microfilaments in response to t-BHQ. Also, depolymerization of actin causes actin-bound Nrf2 to translocate into nucleus and stimulates binding of the complex to ARE for t-RGST2A induction.

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

W. C. Dooms,1 P. B. Tchougouwou1, A. B. Inahate1 and E. Shen.1 Molecular Toxicology Research Laboratory, Jackson State University, Jackson, MS and 2Xenomer Research Laboratory, Xenomer Inc., Boulder, CO.

Pentachlorothenol (PCT) is a biocidal chemical with several industrial, agricultural, and domestic applications. There is accumulating evidence indicating that PCT is highly toxic to humans, with major target organs including the lung, liver, kidneys, heart, and brain. Evidence of PCT-induced mutagenicity and carcinogenicity has also been demonstrated in recent studies. However, little is known regarding its molecular mechanisms of toxicity. This research was therefore designed to assess the cellular and molecular responses of HepG2 cells following exposure to PCT. Cytotoxicity was evaluated using the MTT assay for cell viability, while the CAT-Toxicity (L-) assay was performed to measure the transcriptional activation of stress genes in thirteen different recombinant cell lines generated from HepG2 cells. The cytotoxicity experiment yielded a LD50 value of 234.5±6.6 μg PCP/mL upon 48 hrs of exposure, indicating that PCT is acutely toxic. A dose-response relationship was measured with respect to gene induction. For example, fold inductions of CYP1A1 were 1.0±0.0, 1.0±0.1, 1.3±0.5, 6.3±4.3, and 22.5±3.5 for 0.6, 12.5, 25, and 50 μg PCP/mL respectively. Overall, five out of the thirteen recombinant cell lines tested showed inductions to statistically significant levels (p<0.05). At 50 μg PCP/mL the average fold inductions were 22.5±3.5, 22±8.2, 8.4±1.9, and 12±3.6, for CYP1A1, XRE, HMTIII, c-fos, and GADD153, respectively. These results indicate the potential of PCT to undergo Phase I biotransformation in the liver (CYP1A1, XRE), to cause cell proliferation (c-fos), growth arrest and DNA damage (GADD153), and to influence the toxicokinetics of metal ions (HMTIII). Marginal inductions were recorded for HSP70, CRE, RARE, GADD45, and GADD51. Within the dose range 0.60 μg/mL tested, no significant inductions (p>0.05) were observed for GSTAT, NFκB, and p38RE. (Research supported by Title II Grant No. P031B990006).

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

K.P. Miller and K. S. Ramos. Faculty of Toxicology and Center for Environmental and Rural Health, Texas A&M University, College Station, TX.

BENZO(A)PYRENE (BaP)-induced oxidative stress activates nuclear protein binding to human c-Ha-ras antioxidant/electrophile response element (ARE/EpRE) in vascular smooth muscle cells. The minimal consensus sequence of the ARE/EpRE has been defined as 5′-TGTGAC/NNNGC-3′. Using a 27 base pair (bp) oligonucleotide containing the core c-Ha-ras ARE/EpRE sequence in genomic context (hHras27), two specific DNA:protein complexes of variable complexity were resolved in electrophoretic mobility shift assays in response to BaP and its metabolites, and shown to be of different protein composition. Flanking sequences to the ARE/EpRE core of c-Ha-ras participate in the regulation of nuclear protein complex assembly. The studies presented here were conducted to evaluate the site speci-
ficiency of proteins binding to the c-Ha-ras ARE/Epe core. Nuclear protein binding to hHaras27 was absent upon incubation of nuclear extracts with the same oligonucleotide containing a fully mutated ARE/Epe core region (hHaras27WOC). Low molecular weight proteins (p23 and p25) previously shown to interact with the ARE/Epe core of c-Ha-ras were not detected upon UV crosslinking of nuclear protein to this mutant sequence, implicating these proteins in core sequence binding. UV crosslinked binding of p23 and p25 to an oligonucleotide comprising only the core c-Ha-ras ARE/Epe sequence (HRas 110) could not be competed by 100 bp ARE/Epe core sequences of human NQO, and rat GSTA1 genes, indicating that recognition by these proteins may be unique to the c-Ha-ras ARE/Epe. The only difference in sequence between these ARE/Epe core regions is the "NEN" sequence of the conserved site, suggesting that p23 and p25 bind specifically to the core c-Ha-ras ARE/Epe, and that unrestricted base pairs in the consensus sequence may confer both specificity and uniqueness of protein binding to the ARE/Epe core region. (Supported by NIH grants ES04849 and ES09106 to KSR. KPM is a predotor fellow on NIEHS Training Grant ES07273).

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


Sulfur mustard (SM) is a potent alkylating agent that induces skin vesication after cutaneous exposure. Previous work has revealed that SM induces a significant increase in the production of inflammatory cytokines, including IL-8, IL-6, TNF-α, and IL-1β, in cultured keratinocytes and skin. The p38 MAP kinase signaling pathway is activated in response to cellular stress and has been implicated in the upregulation of cytokines in response to inflammatory stimuli. We investigated whether the p38 pathway mediates inflammatory cytokine upregulation in response to SM exposure. A dose-response study in cultured human epidermal keratinocytes (HEK) revealed that p38 is phosphorylated at SM concentrations that are considered to be vesicating doses (≥100 µM) but not at lower doses, which result in DNA damage but not vesication. A time course at the 200 µM exposure revealed that p38 phosphorylation is induced by 15 minutes post-exposure, peaks at 30 minutes and is sustained at peak levels until 8 hours post-exposure. Examination of MKK3/MEKK6, which are two closely related kinases that activate p38, revealed a slightly faster rate of phosphorylation of MKK3/MEKK6 compared with phosphorylation of p38. As a result, the SM-exposed HEK culture media for cytokines revealed that exposure to 200 µM SM increased IL-8, IL-6, TNF-α, and IL-1β beginning at two hours post-exposure, but had little effect on IL-10 or IL-12(p70). When cells exposed to 200 µM SM were treated with the p38 inhibitor SB203580, the levels of IL-8, IL-6, and TNF-α were decreased approximately 80% and the levels of IL-1β were decreased ~60% when compared with cells that were not treated with SB203580. These results show that the p38 MAP kinase signaling pathway is an important mediator of SM-induced cytokine production and suggest that directly targeting this signaling pathway with pharmacological inhibitors may alleviate the profound inflammatory response elicited by cutaneous SM exposure.

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


The retinoblastoma tumor suppressor gene product (pRB) controls the cell cycle by repressing transcription of genes necessary for S-phase entry, interacting with the p53 apoptosis pathway, and facilitating terminal differentiation. Recent work on mammals has demonstrated that pRB interacts with the aryl hydrocarbon receptor (AhR), a member of the bHLH-PAS family of transcription factors. The AhR mediates the toxic effects of TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) and related compounds, but the endogenous ligand and the physiological role of the AhR have yet to be revealed. The Atlantic killifish Fundulus heteroclitus expresses two paralogues of the AhR (AhR1 and AhR2), whereas mammals express only one AhR. It is likely that the pleotropic functions of the one mammalian AhR are partitioned into the two fish paralogues. We hypothesized that Fundulus AhR1 and AhR2 differ in their ability to interact with pRB and regulate the cell cycle. In support of this hypothesis, Fundulus AhR1 contains the LCXCE-motif and the glutamine-rich region that interact with mammalian pRB, whereas Fundulus AhR2 has neither pRB-binding region. We report here the cDNA cloning of Fundulus RB by RT-PCR with degenerate primers and RACE. The Fundulus RB cDNA (2739 bp) encodes a 913 amino acid protein with a predicted weight of 103.5 kDa. The Fundulus pRB amino acid sequence is 50% and 75% identical to the human and medaka pRB sequences, respectively. The A and B pockets (combined of Fundulus pRB are 67% and 81% identical to human and medaka pRB, respectively, and the putative LXXCXE-binding backbone is absolutely conserved among these three proteins. Experiments are underway to test interactions between pRB and the two killifish AhR paralogues. Supported by NIH Grants 1F32ES05935, ES07381 (Superfund), and ES06272, and by a grant from the Donaldson Charitable Trust.

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


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

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

J. M. Mitchell, S. McPherson³, U. Menga³, T. Gregson³ and J. Tigner³. NDSE, Purdue Pharma, Ardeley, NY. ³Toxicology, CIBER, Montreal, PQ, Canada and ²Toxicology and Pathology, Madaus, Köln, Germany.

Senna, a known laxative derived from plants, was administered by oral gavage to Sprague-Dawley (CD-RoSD1) rats once daily at dose levels of 0, 100, 300, 750 or 1500 mg/kg/day for up to 13 consecutive weeks followed by an 8-week recovery period for selected animals. Dose and treatment-related clinical signs included: increased skin and nasal lesions (abnormal modified lymphocytes) in the heads of all rats exposed to Senna. No changes were seen in any of these groups. At both the terminal and recovery necropsies, an increase in absolute and relative kidney weights (relative to both body and brain weights) was observed for both male and female animals receiving 750 and/or 1500 mg/kg/day. Dark discoloration of the kidneys was observed in animals receiving 300 mg/kg/day or above. Histopathological changes following 1.5 weeks of treatment were seen in the kidneys of animals receiving 300 mg/kg/day and above (slight to moderate, tubular basophilic and tubular pigment deposits). In addition, for all treated groups, minimal to slight hyperplasia in the colon and rectum and minimal hyperplasia of the squamous epithelium of the stomach were recorded. Following 8 weeks of recovery, with the exception of the brown pigmentation in the kidney, most pathological findings were not evident. Thus, the biochemical and histopathological changes seen after 13 weeks of treatment with Senna, significantly reversed following 8 weeks of recovery.

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

H. D. Stockmann-Juvala and M. M. Savolainen. Dept of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Helsinki, Finland.

Fumonisin B1 (FB1) is a toxin produced by the fungus Fusarium verticillioides (= F. moniliforme). FB1 is commonly present in Fusarium infected corn, but it is thought that FB1 also could be present in buildings with moisture and mold problems. FB1...
is known to cause for example leukoencephalomalacia in horses, pulmonary oedema in pigs and liver cancer in rats. The aim of this study was to try to clarify the mechanisms whereby FB1 affects neuronal and glial cells. Four different cell lines were used: human SH-SY5Y neuroblastoma cells, human U-118MG glioblastoma cells, mouse GT1-7 hypothalamic cells and rat C6 glioblastoma cells. All cell lines were grown in Dulbecco's Modified Eagle Medium in bottles and exposed to FB1 at concentrations of 0.1, 1, 10, 30 and 100 μM for 0-144 h in 6- or 48-well plates. Changes of intracellular glutathione (GSH) levels, cell viability and caspase-3 activity were measured with fluorescence probes, monochlorobimane, propidium iodide and a fluorescent caspase-3 substrate, respectively. Decreased GSH levels were observed in all cell lines after exposure to 100 μM FB1 for 144 h. In U-118MG, GT1-7 and C6 cells decreased GSH levels were also seen at earlier time points. Cell viability of all cell lines exposed to FB1 (1-100 μM) for 144 h was lower than controls. The viability of cells was not affected at earlier time points. Increased caspase-3 levels were observed in GT1-7 cells exposed to 10, 30 and 100 μM FB1 for 72 h. No caspase-3 activation was seen in the other cell lines. The decreased levels of GSH in all cell lines exposed to FB1 for 144 h indicate that long-term exposure to FB1 may harm neuronal and glial cells, and the lowered cell viability at this same time point confirms this observation. The activation of caspase-3 in GT1-7 cells suggests that cell death may occur by apoptosis in this cell line. Supported by the Academy of Finland, Finnish Research Programme on Environmental Health.

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


Kava kava powdered extract (kava) is an herbal supplement that has been selected for toxicological evaluation by the NIH. A method was validated for the analysis of kava in corn oil formulations. Next kava and corn oil doped with kava were extracted under ambient conditions and the extracts were analyzed using an isocratic HPLC method with UV detection at 220nm. A method validation was performed over a dose range of 5-500 mg kava/mL corn oil. Solvent standards containing between 5 and 500 mg kava/mL were extracted by sonication for 60 minutes at ambient temperature with 50 mL of methanol, corresponding to an analytical concentration of 0.1 to 10 mg kga/mL methanol. Spiked corn oil standards containing 5-500 mg kava/mL corn oil were extracted by sonication for 60 minutes at ambient temperature with 50 mL of methanol, corresponding to an analytical concentration of 0.1 to 10 mg kga/mL methanol. Extracts were then analyzed in the same manner. All samples were diluted to a final analytical concentration of 0.1 mg kva/mL methanol. Kava, one of the major kavalactones, was used to prepare quantitation standards (337 mg/mL) to determine the weight percentages of kavain in kava solvent and spiked corn oil standards. The kavain quantitation standards proved to be linear (r=0.99999) and accurate (%RE ≤ 2%). The extraction of kavain from kava solvent and spiked corn oil standards proved to be linear (r=0.99999) and accurate (%RE ≤ 2%). The average % kavain extracted from the kava in the solvent standards was 6.61 ± 0.11 (n=10). The average % kavain extracted from the kava in the spiked corn oil standards was 6.65 ± 0.10 (n=10). The average extraction recovery for kavain from corn oil doped with kava was 100.8 ± 1.5%.

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

J. M. Benson, T. S. Poet and D. G. Badger, Toxicology, Loselace Respiratory Research Institute, Albany, NY, NM, 3Chemical Dosimetry Group, Battelle Pacific Northwest Laboratories, Richland, WA, and 4Center for Marine Science Research, University of North Carolina, Wilmington, NC.

Kasrna brevis produces a series of neurotoxic cyclic polyethers known as brevetoxins (PBtxs). Recent evidence suggests that the respiratory tract, hematopoietic, and immune systems are potential targets for PBtx toxicity following repeated exposure. We have investigated the comparative cytochrome P450-mediated metabolism of PBtx2 and PBtx3 by Sprague-Dawley (SD) rat, Fischer rat, and human liver (pool of 15 males) microsomes. Brevetoxin 2 (0.25 to 10 μg/mL incubation mixture) was incubated (37°C) with microsomes from each strain and species under conditions of linearity with respect to PBtx concentration and time. Control incubations were conducted with inactivated microsomes. Extent of metabolism was quantitated by determining the loss of PBtx in 1/6 ion in the incubation mixture extracts by LC-mass spectrometry (LCMS). The molecular weights of possible metabolites were also determined by LCMS. The affinity of the cytochrome P450s for PBtx2 (K_i) and maximum velocity of the metabolic breakdown (V_max) were calculated. The V_max (mean ± s.e. n=3) for PBtx2 by metabolism SD rat, Fischer rat, and humans were 1.72 ± 0.42, 2.02 ± 0.47, and 1.15 ± 0.03 nmol/mg/min, respectively. The K_i for SD rat, Fischer rat, and human microsomes were 3.80 ± 0.62, 4.98 ± 1.52, and 4.40 ± 0.55 μM, respectively. Potential metabolites, with (m+1)/z = 759, 783, 807, and 832 were found for both species. Brevetoxin 3 did not appear to undergo metabolism. Results to date suggest that rates of cytochrome P450-mediated metabolism of PBtx 2 by two strains of rats and humans are similar on a mg protein basis. Further work is needed to identify and characterize the toxicities of the metabolites. Research funded under NIH P01 ES059416.

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

A. J. Boussingault, J. Kubanek, J. Wright and D. G. Badger, ’Center for Marine Science, UNC, Wilmington, Wilmington, NC and 4Dept of Biology, Georgia Tech Univ, Atlanta, GA.

Florida red tides are the result of blooms of the unarmoured dinoflagellate Karenia brevis. K. brevis produces 9 different highly potent polyether brevetoxins (PBtxs) that bind with high affinity to site 5 of the VSSC. The relative profile of toxins produced by K. brevis depends on growth phase and strain. These two findings may explain the variable potency of individual red tide blooms. A naturally-produced competitive antagonist for brevetoxins is described. The antagonist is found at low concentrations in rapidly-growing K. brevis cells but increases to 20% of the total toxin biomass in senescent cells. The antagonist has low potency in fish bioassay (1μg/mL (n=10) and 2 μg/mL (n=5) produced no 24 hr deaths), and in 1μg/mL combination with PBtx2 prolonged time until death by 2.5-fold. The antagonist cross-reacts in PBtx2-LDLA, indicating an intact H-K ring. Low resolution ESI gave an M + 1 peak, m/z of 657.5. High resolution FAB mass spectrometry gave a molecular ion, m/z 656.90. Proton NMR of the antagonist indicates that the C-2, C-27 and C-28 hydrogens are no longer unsaturated, and there appears to be additional sites of unsaturation within the molecule. The exomethylene hydrogen unsaturation is also missing, but there appears to be an endomethylene in conjugation with the terminal aldehyde. The structure of the antagonist appears to be similar to the brevetoxin backbone of at least the H-K rings and most likely include the E-K or F-K rings with further points of unsaturation on side chains. The ring activity region is absent. This is the first report of a competitive non-toxic ligand produced by K. brevis, and may explain the rapidly decreasing potency of red tides during their terminal phases in natural environments. It also represents an additional antagonist for nerve membrane study of marine toxins, and joins synthetic antagonists alpha- and beta-naphthyl brevetoxins.

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

V. L. Johnson, Q. He, M. P. Uschokowski and R. C. Sharma, Physiology and Pharmacology, The University of Georgia, Athens, GA.

Silymarin, a mixture of bioactive flavonoids isolated from Milk Thistle (Silybum marianum), has been used for centuries as a natural remedy and is now effectively used in the treatment of liver toxicity and disease in humans. In vitro studies show that silymarin can inhibit the production and damage caused by tumor necrosis factor (TNFα). It is also a potent antioxidant both in vitro and in vivo. These studies suggest silymarin may impact the immune system but little information exists following in vivo exposure. Therefore, we tested the hypothesis that exposure to silymarin will modulate the inflammatory immune response. Male BALB/c mice (6/group) were treated intraperitoneally once daily for five days with 0, 10, 50 or 250 mg/kg of silymarin. Splenic cultures were stimulated with 1 μg/mL of LPS for 6 hrs following which gene expression was analyzed by reverse transcriptase-polymerase chain reaction. Silymarin exposure did not produce any signs of overt toxicity or any changes in relative organ weights. Functional examination of secondary lymphoid tissue revealed that phylloquinone-induced T-lymphocyte proliferation is increased in the lowest dose group only. B-lymphocyte blastogenesis induced by lipopolysaccharide was increased following exposure to 10 and 50 mg/kg of silymarin. Flow cytometric examination of splenic lymphocyte populations showed that the absolute number of CD4 T-lymphocytes was reduced in the 10 mg/kg group only. A concomitant decrease in the CD4+ T-helper cell population was evident in this group. Similarly, the expression of interleukin (IL)-2 and IL-4 were decreased in the 10 mg/kg group only. In contrast, expression of TNFα, inducible nitric oxide synthase, IL-1β and IL-6 mRNA were increased dose-dependently. The results indicate that silymarin exposure to silymarin results in suppression of T-lymphocyte function at low doses and stimulation of inflammatory processes at higher doses. Further studies investigating the effects of silymarin on the immune system are warranted. (Supported in part by NIH TW010099)
223 EFFECTS OF SILYMARIN ON REGIONAL BRAIN NEUROTRANSMITTER LEVELS IN BALB/c MICE.

M. F. Osuchowski, V. L. Johnson*, Q. He* and R. P. Sharma*. *Animal Anatomy, University of Warmia and Mazury, Olsztyn, Poland and **Physiology and Pharmacology, The University of Georgia, Athens, GA.

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

224 SILYMARIN ALTERS THE EXPRESSION OF CYTOKINES IN MOUSE LIVER.

Q. He*, M. F. Osuchowski, V. L. Johnson*, and R. P. Sharma*. *Physiology and Pharmacology, The University of Georgia, Athens, GA and **Animal Anatomy, University of Warmia and Mazury, Olsztyn, Poland.

Silymarin is a flavonoid mixture extracted from Silybum marianum (St. Mary's thistle) and its main components consist of silybin, isosilybin, silychristin, and silydianin. It has been shown to be protective in various forms of liver diseases including alcohol- and drug-induced problems, and acute and chronic hepatitis. Silymarin may be hepatoprotective due to its antioxidant activity, increase in cellular glutathione content, membrane stabilizing effect, and increase of protein synthesis by RNA polymerase activation. The present study investigated the effect of silymarin on expressions of hepatic cytokines and factors involved in inflammatory reactions and cell growth. BALB/c mice (6-7 weeks old) were randomly divided into 4 groups of 6 mice each and treated with 5 daily intraperitoneal injections of silymarin at 0, 10, 50, or 250 mg/kg body weight. Cytokine gene expression was analyzed by reverse transcriptase-polymerase chain reaction. Silymarin treatment resulted in dose-dependent but insignificant decreases in plasma alanine aminotransferase and aspartate aminotransferase. Silymarin caused dose-dependent increases of the transforming growth factor beta 1 (TGF-β1) and c-myc expression in liver: significant increases were observed at 250 mg/kg group. Dose-related significant increases were noted in the expression of hepatocyte growth factor and interferon-γ. Silymarin treatment did not change the expression of tumor necrosis factor-α or class II major histocompatibility complex in the liver. It has been reported that TGF-β1 is fibrogenic in liver, and c-myc can induce cell proliferation or apoptosis under different conditions. Results suggest that long-term, high dose administration of silymarin may prevent or induce hepatic damage due to a persistent disruption of TGF-β1 and c-myc expression. (Supported in part by NIH TW01009)

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


There is an increase in the application of natural products in cosmetics industries worldwide. In an attempt to assess the cyotoxicity of the local Malaysian plant products, an in vitro cytotoxicity study was carried out using a mammalian Vero cell model. Vero monkey kidney cells. The uptake of the vital dye neutral red (NR) into the lysosomes/endosomes and vacuoles of living cells were used as a quantitative indication of cell number and viability. Indigenous cosmetic ingredients of two essential oils of Citronella (Cymbopogon narudus, also known as serai wangi) and Tea tree (Melaleuca sp.), and a commercially available surfactant namely Plantacare 1200 were assessed using this method. The IC50 value, defined as the concentration of compound that caused a 50% reduction in the uptake of NR when compared to the controls was determined. Our results show that Citronella oils IC50 value is 108.6 ± 35.2 mg/ml which is toxic to tea tree oils IC50 of 113.7 ± 6.3 mg/ml. The IC50 of Plantacare 1200 is 70.6 ± 4.5 mg/ml, which is relatively more toxic than our two indigenous plant ingredients. In conclusion, we have demonstrated that the cytotoxicity of Citronella and Tea tree oil extracted from our local sources are relatively safe as compared to the commercially available surfactant which is used in the formulation of the cosmetics. (This study was funded by IRPA grant no. 09-01-01-2021)

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

J. Kang, Y. Jeon*, H. Kim* and K. Yang*, *Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon, South Korea, **Department of Pharmacology, Chonnam National University School of Medicine, Kwangju, South Korea and Biotechnology Evaluation Laboratory, Korea Research Institute of Bioscience and Biotechnology, Taejon, South Korea.

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

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

E. A. Merrill*, R. A. Clevell*, I. M. Geisthart* and P. J. Robinson*, *OptiTech Corp., Wright-Patterson AFB, OH, **Geo-Centers, Inc, Wright-Patterson AFB, OH, and ***ManTech Environmental Technology Inc., Wright-Patterson AFB, OH and ****ManTech Environmental Technology Inc., Wright-Patterson AFB, OH.

A preliminary model was developed to account for upregulation of thyroid iodide (I) uptake after subchronic perchlorate (ClO4–) exposure. A previous physiological (base line) pharmacokinetic (PBPK) model was capable of predicting ClO4– induced inhibition of I uptake at the sodium iodide symporter (NIS) after acute perchlorate exposure, but did not account for upregulation by thyroid stimulating hormone (TSH). Nasal thyroid follicular Vmax values (approximately 1.5 and 0.7 mg/hg tissue for I and ClO4–, respectively) were estimated by visually fitting radiolabeled uptake data at baseline TSH levels (prior to ClO4–). TSH upregulates the thyroid by stimulating an increase in NIS synthesis, thereby increasing iodide transfer into the thyroid. In this model the increase in NIS as a result of prolonged perchlorate exposure is estimated by adjusting the thyroid follicular Vmax. A mathematical equation was derived that is capable of expressing changes in Vmax, which are dependent upon circulating serum ClO4– levels and perchlorate’s affinity to NIS. In addition, the “upregulated” Vmax is expressed as a time dependent function. Utilizing this method, the model predicts the time and dose dependent behavior of I uptake in the rat thyroid during 14 days of ClO4– exposure.

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

R. S. De Weck* and R. W. Setser. USEPA/ORD/NHEERL, Research Triangle Park, NC.

5-Fluorouracil (5-FU) is a widely used chemotherapeutic drug for the treatment of solid tumors, and is a known teratogen in a variety of test species. 5-FU has an extensive literature and was chosen as a prototypic toxicant to construct a biologically
developed in this study represents an original, mechanistic tool for simulating the lipid concentrations and pharmacokinetics of HIVOCs without the use of tissue blood partition coefficients.

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

A. Archer and K. Krishnan. Groupe de recherche en toxicologie humaine (TOXHUM), Université de Montréal, Montréal, PQ, Canada.

In PBPK models, the tissue uptake of non-ionized organic chemicals (NIOCs) is described as a perfusion-limited process. Conceptually, this process reflects passive diffusion of chemicals across capillary membranes according to Fick's law. For calculating chemical flux as per Fick's first law, diffusion coefficient (D), membrane thickness (T), and area of the membrane (A) should be known. In physiological models, chemical flux is calculated with the knowledge of tissue mass area product, i.e., PA(D-xA)/V as well as tissue blood flow rate (Q) according to the equation: PA(D-xA)/V = Q. When the PA value is very large compared to Q, the latter (i.e., Q) is used as the determinant of chemical flux between tissues and blood. Actually, PBPK models for NIOCs use Q as the determinant of chemical flux without any knowledge of PA. The objective of the present study was to develop an approach for predicting PA for various tissues of human PBPK models, and to compare them with Q, using styrene as the example. For computing the values of PA of chemicals, the D values were obtained using the relationship, log D = 3.20244 log MW + 1.974, whereas the T for capillaries was set to 0.001 cm and the value of A (x1000 cm) for various tissues in various species were obtained following a review of the literature (liver, 98; adipose tissues 200; slowly perfused tissues: 402; richly perfused tissues: 300). The results of this study suggest that, for chemicals with PA values greater than 230, 310, 980 and 1100 L/hr, Q is the limiting factor of tissue uptake in the adipose tissue, slowly perfused tissues, liver and richly perfused tissues. Further, using the tissue blood flow rates calculated for styrene, the present study showed that the perfusion-limited PBPK model is in fact appropriate for this chemical. This study represents the first attempt to predict PA values and provides the basis for determining the appropriateness of the use of perfusion-limited description of tissue uptake of chemicals in PBPK models.

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

K. Sokoloff and K. Krishnan. Groupe de recherche en toxicologie humaine (TOXHUM), Université de Montréal, Montréal, PQ, Canada.

Tissue partition coefficients (P_{i}) correspond to the ratio of solubility or equilibrium concentration of chemicals in different matrices. A common default approach in PBPK modeling is to assume that P_{i} is species-invariant. Neither the validity of this assumption nor the magnitude of the interspecies differences in P_{i} is known. The objective of the present study was to estimate the magnitude of interspecies differences (rat versus human/mouse versus human) in tissue partition coefficients (PCs) of organic chemicals. The approach involved the calculation of the species-specific P_{i} for several chemicals with n-octanol-water PC (P_{i} values ranging from 0.01 to 10000), and dividing the human P_{i} with the animal P_{i}. The computation of chemical-specific P_{i} required data on lipid and water contents. These data for the various rat and human tissues were obtained from the literature. The results of this study suggest that the maximal value of human/rat ratio is about 3 for muscle and liver PCs whereas this ratio is near unity for adipose tissue and liver PCs. The calculated maximal value of human/mouse ratios for muscle and liver PCs are 1.4 and 0.6, respectively. These results suggest that adipose tissue and liver PC determined using rat tissues can be used directly in human PBPK models whereas the muscle and liver PCs should preferably be determined using human tissues. Alternatively, if the P_{i} of a chemical is known in one species, the facotr derived in the present study can be used to estimate the animal-human adjustment factors applicable for the tissue-air PCs. The present study for the first time has estimated the magnitude of rodent-human differences in tissue partition coefficient of organic chemicals based on tissue composition data.

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

M. Beliveau and K. Krishnan. Groupe de recherche en toxicologie humaine (TOXHUM), Université de Montréal, Montréal, PQ, Canada.

The metabolism rate constants (V_{max}, maximal velocity for metabolization; K_{m}, Michaelis affinity constant) of alternatively the hepatic extraction ratio (E = CL_{UM}/[Q1 + CL_{UM}] where Cl_{UM} = V_{max}/K_{M} and Q1 = liver blood flow rate) for PBPK
modeling are obtained from *in vitro* or *in vivo* studies. Validated *in silico* approaches for predicting \( V_{m} \), \( K_{m} \), or \( E \) are yet to become available. The objective of this study was to develop an approach for estimating the hepatic extraction ratio \( (E) \) using the steady-state blood concentration \( (C_{b}) \) of chemicals in humans. The approach consisted of using experimental values of \( C_{b} \) of toluene \((T)\), n-xylene \((N)\) and ethylbenzene \((E)\) in order to estimate their apparent blood-air partition coefficient \( (P_{a}) \). Knowing the fraction of blood flow to the liver \( (Q) \) and the blood-air partition coefficient \( (P_{a}) \) of the chemical, it was possible to estimate \( E \) \((0.75 \pm 0.05) \) for \( 0.75 \pm 0.05 \) according to the following algorithm: \( P_{a} = P_{a}(1+QF_{r}) \) derived from the PBPK model. The relation between the \( P_{a} \) and the \( P_{a} \) of n-xylene \((19) \) volatile organic chemicals was also investigated. A log-linear relationship \( \log P_{a} = 0.6807 \) \( P_{a} = 0.6807 \) \( \log 0.1 - 0.1167 \) was found to adequately reflect the qualitative relationship between \( P_{a} \) and \( P_{a} \) and this relationship indicates the feasibility of predicting \( E \) from knowledge of \( P_{a} \) of VOCs. The results of this study demonstrate the adequacy of using blood concentration data and \( P_{a} \) for estimating \( E \) for human PBPK models of VOCs.

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

L. K. Potter, T. H. Bane, M. V. Evans, and R. A. Albane.

*Curriculum in Toxicology, UNC, Chapel Hill, NC; TED, NIEHSL, ORD, USEPA, Research Triangle Park, NC; CRC, NC, USA; Brooks AFB, San Antonio, TX.*

Large numbers of xenobiotics are known to accumulate in fat tissue, including many organochlorine pesticides, PCBs, dioxins and volatile organic compounds. This accumulation in the fat has a major effect on the overall pharmacokinetics of such compounds, including blood and target tissue tissue concentrations. The purpose of this research was to develop a PBPK model based on the disposition of the fat-accumulating solvent TCE, with a focus on better describing fat concentrations. Preliminary efforts indicated that the standard PBPK models were not able to capture the dynamics of TCE concentrations in the fat tissue of adult male Sprague-Dawley rats exposed to 50 ppm TCE for 4 hours (unpublished data). Moreover, the assumptions of the standard PBPK models were inconsistent with the observed changes in fat cell size, lipid distribution, and blood flow within the fat depot. These physiological characteristics and the preliminary modeling results led to the development of a specialized compartmental model for TCE in fat tissue based specifically on the physiology. This spatially-varying model included capillary, interstitial fluid and fat cell subcompartments that were based directly on the structure of fat tissue. This compartmentalized fat model was developed and tested for its validity. The model predicts the compartmentalization of TCE within the fat tissue accurately. In summary, a compartmental-fat model is a promising tool in PBPK modeling for accurately describing the disposition of fat-accumulating compounds. (This abstract does not reflect EPA policy).
ESTIMATING PREVIOUS SERUM TCDD CONCENTRATIONS IN AN OCCUPATIONALLY EXPOSED COHORT USING A PBPK MODEL.

O. A. Dankovic and M. E. Andersen. † Risk Evaluation Branch, NIOSH, Cincinnati, OH and *Dept. of Environmental Health, Colorado State University, Fort Collins, CO.

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

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

G. Crum et al. † R. Sarangapani, † J. Requinet, † H. J. Clewell and M. E. Andersen. † Toxicology, Bridgewood, NJ, † K.S. Crump Group, Research Triangle Park, NC, † K.S. Crump Group, Raritan, NJ and † Dept. Environmental Health, Colorado State U., Fort Collins, CO.

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

ROUTE-SPECIFIC DIFFERENCES IN DISTRIBUTION CHARACTERISTICS OF OCTAMETHYLCYCLOTETRAISOXANE IN RATS: A PHYSIOLOGICAL MODEL BASED ANALYSIS.

K. P. Ploetz, R. Sarangapani and M. E. Andersen. † Dow Corning Corporation, Midland, MI; † The K.S. Crump Group, Research Triangle Park, NC, and † Colorado State University, Ft. Collins, CO.

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

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

M. B. Reddy, † L. D. Dobrey, † M. J. Ueili, † P. E. Morrow, † K. P. Ploetz and M. E. Andersen. † Quantitative & Computational Toxicology Group, CETF, Colorado State University, Ft. Collins, CO; † University of Rochester, Rochester, NY and † Dow Corning Corporation, Midland, MI.

D4, a lipophilic, volatile liquid, is used as a reaction intermediate and is found in industrial and consumer products. Workers and consumers may be exposed to D4 by inhalation or dermal routes. A human inhalation pharmacokinetic (PK) study (Toxicology Sciences, 44:206-13, 1998) and a physiologically based pharmacokinetic (PBPK) model for D4 inhalation in rats (Toxicology Sciences, 60:214-31, 2001) were recently published. The rat PBPK model showed that D4 is eliminated rapidly from the body after exposure due to its low bloodair partition coefficient, Pb (~1), and its relatively high metabolic clearance. Some D4 was present in blood in a form that was not readily exhaled, presumably bound to lipids. From the human PK study, data from 12 subjects exposed by inhalation to 10 ppm D4 for 1 h with alternating periods of rest and exercise were analyzed with the PBPK model. The human data required a Pb of about 0.9. Human metabolism and fat partitioning constants remain to be determined more accurately (they could not be estimated from the data). When values for these parameters were based on those of the rat study, the model matched observations in the human PK study very well. The PBPK model results were also consistent with the decreased retention with increased alveolar ventilation seen in humans. While the human data set is less extensive than the rat data set, similar PK behaviors were noted including rapid clearance by exhalation and longer persistence in plasma than expected based on the Pb. The rate of metabolism of D4 in the human model in PBPK modeling of D4 inhalation kinetics in rats appear valid for human kinetics of D4. Thus, a similar model structure, with a key feature of D4 sequestration in blood lipids, is required in both species. Supported in part by Silicones Environmental, Health and Safety Council of North America and by NIHES T32 ES07231 for M. Reddy.

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

L. D. Dobrey, † K. P. Ploetz, † M. B. Reddy and M. E. Andersen. † Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Department of Environmental Health, Colorado State University, Fort Collins, CO and † Health and Environmental Sciences, Dow Corning Corporation, Midland, MI.

Gas uptake methods have been successfully used to assess the metabolism of a wide variety of volatile organic chemicals. The technique works best with compounds of moderate blood-air and relatively low fat-blood partitioning, and little propensity to adsorb onto chamber surfaces. We extended these techniques to evaluate the in vivo metabolism of hexamethyldisiloxane (HMDS), a low molecular weight volatile siloxane. The studies utilized a static atmosphere closed chamber system designed by Fisher and colleagues (Arch. Toxicol. 42: 123-136, 1979). Soda lime, used to prevent accumulation of exhaled CO2 in the system, adsorbed a substantial part of HMDS thus limiting the interpretation of the kinetic data. Soda lime was replaced
by 3.5 N NaOH providing acceptable background loss rates of less than 2% per hour. After resolving these technical issues, a series of gas uptake experiments were initiated to evaluate the metabolism of HMDS. In each experiment, two rats (Fischer 344, male, 8-9 weeks old) were exposed to initial concentrations of HMDS ranging from 500 to 5000 ppm and the chamber concentration was monitored over a period of 8 hr. The mass of HMDS from the chamber atmosphere were modeled with a four-surface PBBK model. Excess samples were obtained incorporating a single saturable metabolic pathway (Vmax=98 µM/hr; Km=86 µM) restricted to the liver. Blood-air and fat:blood partition coefficients were 1.5 and 230. The availability of a PBBK model for HMDS will provide further means to quantitatively characterize the retention, distribution, metabolism and elimination of this material, and support risk assessment extrapolations across dose, dose rate, and species. Supported in part by Silicones Environmental, Health and Safety Council of North America.

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PBBK MODELS OF KEPONE, MIREX, HEXAFLUOROACETONE AND ACETONE: SUGGEST BINDING TO HEPATIC TISSUE

C. C. Belfiore, M. E. Andersen, M. Lochtnay, O. S. Lochtnay and R. S. Yang, CETF, Quantitative and Computational Toxicology Group, Colorado State University, Fort Collins, CO.

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

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DOSE DEPENDENT RATES OF MANGANESE (Ma)

TAKING AND BILIRINARY ELIMINATION:

COMPARTMENTAL ANALYSIS OF DENTAL AND GAVAGE DOSING STUDIES IN THE RAT


Tissue concentrations of the essential element Mn are regulated predominantly by control of gastrointestinal uptake and bilirinary elimination. Understanding of the comparative uptake, elimination and tissue distribution of Mn following oral or inhalation exposure to Mn is central to the development of quantitative tools for risk assessment of inhaled Mn. A three-compartment pharmacokinetic model consisting of the gut lumen, gut tissues and the liver was used to examine dose dependencies of Mn for absorption and elimination by simulating dietary or gavage exposure studies in rats. Fitted rate constants provided excellent fits to true and apparent uptake of Mn following dietary exposure at concentrations of 1.5 µg/g and 100 µg/g. The first order rate constants for uptake decreased by a factor of 20 and the first order elimination rate increased approximately 100 fold across the dose range, the rate constant for elimination tending toward saturation at dietary levels greater than 35 µg Mn. The model was then compared with two additional dietary exposure studies, and provided good fit to reported bile elimination rates and liver concentrations. Combined dietary (65 µg/g) and oral gavage (0.2-10 mg Mn) appeared to exceed the capacity of uptake control, resulting in compensatory increases in elimination. These empirical representations of the important control processes for regulation of tissue Mn concentrations are necessary for incorporating dose-dependencies into physiologically based pharmacokinetic models describing tissue disposition following combined oral and inhalation exposure to Mn or Mn-containing products of combustion. This work was supported by Ethyl Corporation.

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

M. H. Lumpkin, J. L. Fisher, J. L. Heuchert and C. F. Dallas, Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA and Environmental Health Science, University of Georgia, Athens, GA.

Exposure to trichloroacetic acid (TCA) is widespread, as a by-product of drinking water purification or as a metabolite of chlorinated solvents such as trichloroethylene. TCA has been shown to cause liver tumors in mice and is considered a probable human carcinogen. Physiologically-based pharmacokinetic (PBBK) modeling has important utility in examining species-related differences in chemical metabolism and distribution. A better understanding of the role of plasma protein binding for important metabolites like TCA and its influence on their bioavailability to liver and other tissues is needed for human risk assessment. However, there is a paucity of data. Mathematical expressions for the binding of TCA to human plasma proteins were incorporated into PBBK models for each species. Information on the free fraction proved useful in making more accurate model predictions of liver bioavailability over a wide range of TCA plasma concentrations. Comparisons of species-specific differences in liver bioavailability as a function of TCA plasma concentration were thus demonstrated to be feasible, which can have significant utility in using PBBK modeling to assess human health risks. (This research was funded by Department of Energy Grant # DE FG02 98CH10902)

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

M. G. Zagler, G. T. Tian and F. M. Schlessinger, ERDT, DIT, Centers for Health Research, Research Triangle Park, NC, and Mathematics, North Carolina State University, Raleigh, NC.

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

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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF DICHLOROACETIC ACID

R. D. Sonner and L. B. Schultz, Battelle PNL, Richland, WA and Battelle PNL, MSIS, Squam, WA.

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

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water with the eventual goal of eliminating DCA from drinking water under stage 1 regulations. Thus, understanding and predicting the pharmacokinetics of HAs associated with drinking water disinfaction is critical to assessing associated risks to the public. Recently, we characterized the low dose pharmacokinetics and oral bioavailability of DCA in F344 rats. Important features of DCA kinetics are the pronounced non-linearity in its elimination and the complex plasma-time profiles observed after oral dosing with secondary peaks appearing well after the initial absorption phase. The secondary peaks were not caused by enterohepatic recirculation ( bile flow independent). The elimination of DCA is controlled primarily by liver metabolism, mediated through a novel class of GST isoenzyme, GST-zeta. An interesting feature of the GST-zeta pathway is the loss in enzyme activity and decreased metabolism that occurs following exposure to DCA. To aid in the risk assessment of HAs, we have been developing a physiologically based pharmacokinetic (PBPK) model for DCA that incorporates key aspects of DCA disposition identified from past experimental studies using both naïve rats and rats treated to deplete GST-zeta enzyme levels. The DCA model to be presented includes a multi-compartment description of the gastrointestinal system to account for the observed discontinuous absorption of DCA. The model also describes liver metabolism using the dispersion model that is better able to account for the saturable metabolism and resultant non-linearity in DCA elimination. Model predictions were validated against previously characterized plasma-time profiles for DCA and selected tissue concentration-time profiles obtained for this study. Supported by EPA STAR grant R825954.

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


Chlorobenzene is an industrial solvent used in the manufacture of pesticides, resins, inks, and paints. To better understand the dose-dependent and route-specific kinetics of chlorobenzene, this work focused on development and validation of a physiologically-based pharmacokinetic (PBPK) model to describe the absorption, distribution, metabolism and elimination of chlorobenzene in rats. Partition coefficients were experimentally determined in rat tissues and blood samples using a via equilibration technique. These solubility ratios were in agreement with previous reports. The in vivo metabolism of chlorobenzene was evaluated using groups of 3 F344 male rats exposed to 80-675ppm chlorobenzene in a closed, recirculating gas uptake system. Michaelis-Menten metabolic constants were optimized from the family of gas uptake curves using the PBPK model, and indicated a high affinity (Km) and high capacity (Vmax) for chlorobenzene metabolism. At the highest chamber concentration, the uptake curve could not be modeled without the addition of a first-order (Kd) metabolic pathway. Pretreatment of animals with pyrazole, an inhibitor of oxidative microsomal metabolism, completely abolished uptake. The completed PBPK model was validated against real-time exhaled breath data collected from rats administrated 400mg/kg chlorobenzene in corn oil by oral gavage or intraperitoneal (IP) injection. Absorption rates were optimized from the exhaled breath profiles of the animal per exposure route, and used to simulate the remaining data sets. Development of a comprehensive PBPK model for chlorobenzene in rats is the first step toward future extrapolations to apply to humans (Supported by the US Department of Energy under Contract DE-AC06-76RL01830).

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


Methyl ethyl ketone (MEK) is an industrial chemical commonly encountered in a wide variety of paint products. A PBPK model to describe the absorption, distribution, metabolism and elimination of methyl ethyl ketone (MEK) in rats was developed. Partition coefficients were experimentally determined in rat tissues and blood samples using a via equilibration technique. These solubility ratios were in agreement with previous human-based estimates that MEK is uniformly soluble within all tissues. Both saturable (Km = 0.63 mg/L; Vmax = 5.44 mg/hr/kg body weight) and first-order (Kd = 4.11/hr) metabolic rate constants for rats were derived from the optimization of a series of in vivo gas uptake curves conducted at various initial chamber concentrations. Pretreatment with pyrazole, an inhibitor of oxidative microsomal metabolism, decreased the slope of the uptake curve, but did not abolish metabolism. Optimal model fit to the gas uptake curve from pyrazole-pretreated animals was achieved with Km increased 50 times the value determined from naive rats. The completed PBPK model was validated against real-time exhaled breath data collected from rats administered an intravenous (IV) injection of MEK. Model simulation of the IV-treated animals required alveolar ventilation to be reduced 30% in order to match the data. Exhaled breath profiles from animals treated with MEK by oral gavage or intraperitoneal (IP) injection exposures were simulated, and absorption rates determined. Development of a comprehensive PBPK model for MEK in rats is the first step toward future extrapolations to apply to humans (Supported by NIOSH 1-R01 OH03658-01A2).

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


Perchlorate (ClO4-) disrupts endocrine homeostasis by competitively inhibiting the transport of iodide (I-) into the thyroid. Therefore, the possibility of developmental effects from ClO4- induced iodide deficiency during gestation is cause for concern. Fetal development relies on maternally supplied I- for hormone synthesis within the fetal thyroid. Though ClO4- passes through the placenta, the extent of inhibition in the fetus is unknown. A physiologically-based pharmacokinetic (PBPK) model was developed in the pregnant rat to predict ClO4- exposure and the resulting effect on iodide kinetics. Tissues with active uptake were described with Michaelis-Menten (M-M) kinetics to simulate saturable transport. Physiological and kinetic parameters were obtained from literature and experiment. Systemic clearance, placental, fetal transport and M-M uptake parameters were obtained by fitting simulations to experimental data in the pregnant and fetal rat. The rat model is able to predict iodide kinetic data in the dam and fetus over 5 orders of magnitude (0.36 to 53,000 ng/kg day -1), ClO4- distribution over 3 orders of magnitude (0.01 to 10 mg/kg day -1), and inhibition of maternal thyroid uptake. The successful rat model served as the basis for a description of the corresponding human kinetics. Physiologically based species and tissue kinetic differences were obtained from literature and previously developed adult male rat and human ClO4- models. Kinetic parameters were scaled allometrically from the rat gestation model, except the parameters with significant species differences, which were obtained from the male human model and literature. The resulting model successfully describes maternal, fetal, placental and thyroid, as well as fetal thyroid, kidney, stomach, serum and total fetal iodide in the human from several studies through gestation (weeks 6 to 40).

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


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

J. M. Robile and T. W. McMillen and M. E. Anderson. Environmental Health, Colorado State University, Fort Collins, CO.

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

253 THE EFFECTS OF SMOKING ON HUMAN HEALTH.


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

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

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

Cigarette smoke is a potent immunosuppressive agent that completely blocks T and B lymphocyte responses in the lungs. We have identified several compounds in both the tar and gas phases that inhibit distinct events associated with T cell activation and proliferation. Hydroquinone (HQ), catechol and phenol are among the three major phenolic components in cigarette tar. They are present in cigarette smoke at concentrations that are directly proportional to tar content. Hydroquinone and catechol inhibit lymphocyte proliferation by interfering with two different stages of the cell cycle. When added to testing T cells prior to mitogenic activation, they permit many early activation events, such as IL-2 production, expression of CD25 and CD69. However, they also impair thymocyte proliferation from a G0/S phase of the cell cycle. This phenomenon is associated with inhibition of both the G0/S and transferrin receptor expression. When added to proliferating lymphocytes, HQ and catechol cause an immediate cessation in DNA synthesis by quenching the tyrosine radical in ribonucleotide reductase. Phenol has no effect on any of these events. Acrolein and crotonaldehyde are thought to react with Cys and β-unsaturated aldehydes present in the gas phase of cigarette smoke. Unlike HQ and catechol, they are present even in ultra-low tar cigarettes. At doses found in a single cigarette, they inhibit the production of several proinflammatory cytokines, including IL-1β, IL-2, TNF-α and IFN-γ. The immunosuppressive effect can be completely abrogated by N-acetylcycteine, suggesting that they function by reacting with thiol groups. Acrolein and crotonaldehyde inhibit cytokine production by blocking NF-kB and AP-1 DNA binding activity, thereby preventing expression of genes that are regulated by these transcription factors.

256 NEUROIMMUNE COMMUNICATION IN NICOTINE-INDUCED IMMUNOSUPPRESSION.

M. L. Supp. Respiratory Immunology, Laddle Respiratory Research Institute, Allsupers, NM.

Chronic exposure of rats and mice to nicotine (NT) decreases the antibody-forming cell response and Con A-induced proliferation of T cells. This immunosuppression is accompanied by impaired antigen receptor-mediated signaling in T cells, including constitutive activation of Ptk and other protein tyrosine kinases. Similar changes in the immune response were also observed after intraeerebroventricular administration of NT, which were reversed by simultaneous administration of the nicotinic acetylcholine receptor (nACHr) antagonist, mecamylamine, indicating that NT affects the immune system via the nACHRs in the central nervous system. The immunosuppressive effects of NT were also seen in adrenalectomized rats, but not in animals pretreated with ganglionic blockers. Thus, the effects of chronic NT exposure on the immune system are independent of the hypothalamus-pituitary-adrenal axis but dependent on the autonomic nervous system. Surgical sympathetic denervation of the spleen or treatment of animals with the muscarinic antagonist, atropine, did not reverse the effects of NT on the immune system. Therefore, it is likely that the parasympathetic but not the sympathetic pathway transmits signals from the brain to nACHRs on T cells. In this regard, our recent data suggest the presence of α7-containing nACHRs on T cells. Interestingly, following chronic NT treatment, animals exhibit enhanced expression of α7 of hairpin in the brain that is kinetically correlated to the induction of immunosuppression. Moreover, unlike the control wild-type mice, IL-1α receptor KO mice did not exhibit immunosuppression in response to NT exposure. These results suggest that the neuroimmune effects of NT are mediated through the parasympathetic pathway and that IL-1α may play an important role in the neuroimmune modulation by chronic NT treatment. Supported in part by grants from the NIH (DA04208, DA04208-1351, HD38222).

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

K. A. Stringer1 and J. M. Freed2. 'Pharmacy Practice, School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO and Allergy and Clinical Immunology School of Medicine, University of Colorado Health Science Center, Denver, CO.

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

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

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

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

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

K. Dong1, J. Tan1, Z. Liu1, T. Tait1, S. M. Velle1 and A. L. Goldin1. *Entomology, Michigan State University, East Lansing, MI; Pharmacology, Pharmacia Corp., Kalamoso, MI; USDA-ARS, Gainesville, FL; and Microbiology and Molecular Genetics, University of California, Irvine, CA. Sponsor: T. Narashibi.

Pyrethroid insecticides are used worldwide to control insect pests of agricultural and medical importance. Pyrethroids exert toxic effects on insects by binding to a unique, but undefined site on the voltage-gated sodium channel and thereby altering sodium channel gating properties. Multiple sodium channel protein mutations have been found in several pyrethroid-resistant insect species. The mutations, located in IS6, IS6, the linker connecting IS4-S5, and the linker connecting the domains I and II, have been shown to reduce the sodium channel sensitivity to pyrethroids. In this talk, I will review the effects of these sodium channel mutations on the gating and pyrethroid-sensitivity of the German cockroach Para sodium channel. In addition, we recently identified two Para alternative splice variants, which differ greatly in their sensitivities to pyrethroids. Further analysis of these two splice variants will reveal additional amino acid residues involved in sodium channel interaction with pyrethroids.

260 INSECTICIDE RESISTANCE: OLD PROBLEMS OR NEW SOLUTIONS?


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

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


The last decade opened the era of neonicotinoids (represented by imidacloprid) as a major new class of highly effective and widely used insecticides for crop protection and veterinary pest control. The favorable selective toxicity of neonicotinoid insecticides for insects versus mammals is not shared by their N-substituted imines, metabolites, and analogs or by nicotine or epibatidine. The same selectivity profile is evident at the target site level, i.e., the insect nicotinic acetylcholine receptor (nAChR) versus mammalian nAChR subtypes (α1, α2, α4 and α7). The insect-selective compounds are not protonated with a nitrogen, cyanoimine or nicotinamide -substituent, and the mammalian-selective compounds are ionized at physiological pH. We propose that the negatively charged tip of the nitro or cyano group interacts with a putative cationic subsite of the insect nAChR. This contrasts with the mammalian nAChR subtypes where the iminium cation of N-substituted imines metabolites or analogs of neonicotinoids or amidopyrine nitrogen of nicotine and epibatidine interacts with the anionic subsite. Further, only minor structural changes in neonicotinoids confer differential subsite selectivity for mammalian nAChRs. Chronic exposure to neonicotinoids, metabolites and analogs up-regulates the α4β2 nAChRs in M10 cells as initiated by receptor-compound interaction and followed by intracellular events.

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

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

Most insecticides are more toxic to insects than to mammals, and the selective toxicity of certain insecticides has been shown to be ascribed to the higher target site insecticide sensitivity in insects than in mammals. The major primary target of pyrethroids is the sodium channel, and the neuronal sodium channel of cockroaches were more sensitive than that of rats. For example, aldrithion is approximately 1000-fold more potent on the cockroach sodium channel than on the rat dorsal root ganglion (DRG) tetrodotoxin (TTX)-sensitive sodium channel. Based on the measurement of the percentages of sodium channels modified by the pyrethroid, Fipronil blocks the GABA receptor, and was more potent on the cockroach GABA receptor than on the rat DRG GABA receptor. Indoxacarb and its metabolite DCJW have been shown to act on multiple target sites. Both blocked the sodium channels with a higher potency in cockroaches than in rats. They acted on the α7-type and α4 β2-type nicotinic acetylcholine (nACh) receptors of rats in a complex manner: indoxacarb stimulated the 0.4 β2 receptor but inhibited the α7 receptor, while DCJW had no effect on the 0.4 β2 receptor with slight inhibition of the α7 receptor. The cockroach α7 receptor, which had characteristics different from either the rat α7 or α4 β2 receptor, was inhibited by indoxacarb. A marked difference was found in the response of GABA receptors: the rat GABA receptor was totally insensitive to indoxacarb or DCJW, whereas the cockroach GABA receptor was strongly inhibited by indoxacarb. In summary, the mechanisms of selective toxicity to pyrethroids, fipronil and indoxacarb reside in the differential sensitivity of target receptors between mammals and insects. Supported by NIH grant NS14143.

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

V. L. Sugden, Insecticide Biochemistry, Arbos CropScience, Frankfurt am Main, Germany. Sponsor: T. Narashibi.

Nicotinic receptors and chloride-channel-gating GABA and glutamate receptors, all members of the cys-loop superfamily of ligand-gated ion channels, are important insecticide target sites. Recent electrophysiological studies on these receptors in insect neurons show the presence of subtypes of each of these receptor types, with large differences in sensitivity to insecticides and other drugs. Additionally, compounds that are strongly inhibited on these receptors, as blockers or channel blockers, can act on more than one type of receptor, such as GABA receptors. For example, the effects of avermectins on GABA receptors were studied long before the inhibitory glu...
timate receptor were recognized as target sites, and more recently, fibrinol, which has long been known to block GABA-gated chloride channels, has been found to also block glutamate-gated chloride channels. Similarly, since mode of action is modulation of nicotinic receptors, has also shown effects on GABA receptors. Thus, a complete understanding of the insect toxicology of these compounds requires an understanding of their interactions with a multiplicity of receptors. New comparative studies of the actions of neonicotinoids, spinosyns and fipronyl on subtypes of nicotinic, GABA and Glutamate receptors help to clarify our understanding of the role of these insecticides on these important target sites, and provide important new insights into resistance mechanisms and insect selectivity.

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

G. P. Daston1 and R. A. Coles2. 1Miami Valley Labs, Pricer & Gambie, Cincinnati, OH and 2Pacific Northwest National Lab, Richland, WA.

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

265 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF PRENATAL GROWTH AND EXPOSURES.


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

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


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

267 PHARMACOKINETIC CONSIDERATIONS IN CHILDREN’S RISK ASSESSMENT.

G. L. Ginsberg, D. Harris3 and B. Sonawane3. 1Dept of Public Health, St. of Connecticut, Hartford, CT; 2Clark Univ., Worcester, MA and 3USEPA, Washington, DC.

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

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

H. A. Bartol3, J. G. Tegeder4, L. K. Potter1 and R. W. Setzer1. 1Pharmaceutical Branch, ETD, USEPA/ORD/HNF/ERL, Research Triangle Park, NC; 2JCP Consulting, Research Triangle Park, NC; and 3Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC.

Questions about the potential for endocrine disruption have focused upon differential sensitivities across life stages and the potential that dose-response behaviors would vary across these periods. Some hormone-mediated changes in gene expression are "organizational" during development (e.g., stabilizing system set points) versus "activational" changes in adults (e.g., altering the current level of expression). Biological modeling of the impact of exogenous compounds on hormonally mediated events should ultimately address both pharmacokinetic and pharmacodynamic differences. Physiologically-based models of the pharmacokinetics of EACs during pregnancy, lactation, through puberty and into adulthood have accounted for factors such as changes in compartment size, blood flow, and serum binding proteins. Use of such models to estimate tissue dosimetry is dependent upon information about the mode of action including the window of vulnerability, the active form of the compound, and the biology of the endogenous signaling system. Models have been constructed for adult and fetal rats, and the central axis function in rats and humans. Models of receptor-mediated processes have described in vivo and in vitro data, e.g. uterine receptor occupancy and positive feedback loops in regulatory phenomena. While such models may help to understand the pharmacodynamics of EACs in adults, understanding their effects during in vitro development may require describing morphogen-like gradients of endogenous hormone.
as well as receptor-mediated responses to circulating hormones. These efforts at pharmacokinetic and pharmacodynamic modeling for EACs of different life stages are designed to assist in evaluating questions of dose-response behavior and cross-species extrapolation. (This abstract has been subjected to Agency review and approved for publication. LKP was funded by the EPA/UNC Toxicology Research Program, Training Agreement CTB27260.)

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

R. J. Kavlock. Reproductive Toxicology Division, U.S. EPA, Research Triangle Park, NC.

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

270 INTRODUCTION TO THIORDEROXINS AND THIORDEROXIN REDUCTASES: CENTRAL ROLES IN TOXICITY AND CANCER.

J.R. Kehler. Pharmacology and Toxicology, The University of Texas at Austin, Austin, TX.

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

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


Thioredoxins are present in all cells and are the main disulfide reduction enzymes with a large number of functions. Reduction of the active site disulfide in thioredoxin (Trx) by NADPH is catalyzed by thioredoxin reductases (TrxR). Mammalian TrxR are very different from the small enzymes present in bacteria and have a very small substrate specificity including the lipid hydroperoxides and selenite. The rat enzyme contains a glutathione reductase scaffold elongated by 16 residues and a C-terminal conserved sequence. Gly-Cys-Sec-Cys, where Sec is selenocysteine. We have expressed the rat enzyme in E.coli by changing the TGA codon for Sec982 to Cys or Ser, or a stop codon. The mutant enzymes were purified to homogeneity in high yield and contained one mol of FAD per subunit. Only the Sec to Cys mutant enzyme was active in reduction of thioredoxin with a 100-fold decrease in Kcat compared to the wild type Se-containing enzyme. The pI-optimum for the Cys mutant enzyme was 9 as opposed to 7 for the Sec-containing wild type enzyme.

272 THIOREDOXIN IN CANCER DEVELOPMENT AND TREATMENT.

G. Powis. Arizona Cancer Center, University of Arizona, Tucson, AZ. Sponsor: J. Kehler.

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

273 REDOX OF THIORDEROXIN IN OXIDANT-INDUCED APOPTOSIS.

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

Two major thiol-containing systems exist that modulate cellular redox balance as well as protect against oxidative stress. One utilizes the tripeptide glutathione (GSH) and the other uses the small protein thioredoxin (Trx). Both systems have similar NADPH-dependent reductases and have overlapping functions and activities. Our recent research shows that the redox state of the GSH pool is maintained over a narrow range in cells and varies in association with cell proliferation, differentiation, redox signaling gene expression and apoptosis. To determine whether the redox state of Trx similarly varies in association with changes in cell function, we have developed a Western blot technique that involves separation of oxidized and reduced forms of Trx based upon the carboxyamidation of thiols. Disulfides do not undergo carboxyamidation and consequently oxidized forms of Trx can be separated from reduced forms by PAGE under native conditions. Each form is detected by a Western blotting using an antibody which detects both oxidized and carboxyamidated forms. Elution of the corresponding bands from the gel and analysis by MALDI-mass spectrometry has been used to confirm the identity of the reduced and oxidized Trx species. The results show that Trx is oxidized during oxidative stress and that redox varies independently of the GSH pool under different growth and toxicologic conditions. Application of the most recent nuclear and cytosolic fractions further shows that redox in the nuclear compartment is distinct from that in the cytoplasm. (Supported by NIH grant ES99047.)

274 REGULATION OF THIORDEROXIN-DEPENDENT PEROXIDASE PERFRODOXIN.

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

Recent evidence suggests that H2O2, produced in response to stimulation of a variety of receptors, serves as an intracellular messenger. Timely elimination of second messengers after completion of their mission is critical for receptor signaling. This would seem especially true for H2O2, which is readily converted to deleterious hydroxyl radicals. Peroxiredoxin (Prx) is a family of peroxidases that exist in multiple isoforms in a variety of cells. The four mammalian members, Prx I, II, III, IV contain two conserved cysteines that correspond to Cys52 and Cys 173 of human Prx I. They exist as a head-to-tail dimer. The oxidized intermediates of Prx I-IV pro-
duced in the presence of H2O2 are dimers containing intermolecular disulfide between the two conserved cysteines and the disulfides are reduced by thioredoxin but not by glutaredoxin or glutathione. Cysteine Prx enzymes (Prx I, II, and IV) participate in receptor signaling by eliminating H2O2. Prx enzymes may participate passively by removing H2O2 molecules that diffuse away from the sites of production or participate actively by being subject to spatiotemporal regulation. Our recent studies suggest that the latter is likely the case. Prx I and II are mainly cysteine but are also found in nuclear fractions. The amount of Prx I and II in the nuclear fraction increases in response to serum stimulation and decreases in response to treatment with H2O2 or UV irradiation. Prx V and PrxL, which contain a conserved site [TPK/RKK] for phosphorylation by cyclin-dependent kinase (CDK), are phosphorylated by several CDKs in cells. Phosphorylation by CDK abolishes peroxidase activity. When experiments were carried out with HeLa and NIH3T3 cells synchronized at various cell cycles, the highest level of Prx I phosphorylation was detected in G2/M phase arrested with nocodazole. When the cells were released from nocodazole block, the levels of Prx I phosphorylation gradually decreased as the number of mitotic cells diminished. These results suggest that Prx I and II play important roles in regulating H2O2 concentration in nucleus.

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

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

This situation will continue to be a growing concern as we attempt to include additional training in the areas of genomics and proteomics in our toxicology programs in order to meet the toxicological needs of the future.

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

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

279 BIOINFORMATICS-BRIDGING A LINK TO TOXICOLOGY.
W. B. Matteo, Predictive & Mechanistic Toxicology, Pharmacis & Upjohn, Inc., Kalamazoo, MI.
In some circles it might seem that any computer application outside of word processing and spreadsheets is "bioinformatics". A proper definition of the word, however, might be "the intersection of computer science, information technology, and biology." While classically, bioinformatics involves analysis of genetic and protein sequence data, it is often used to describe statistical analysis of biological data, database construction and maintenance, database mining, and automated capture of biomedical data. But what does any of this have to do with toxicology? Consider that in five years or less databases WILL exist containing the mRNA and protein responses to select environmental changes (e.g. chemical treatments) for various experimental systems. But what exactly does the increase in a signal for one probe set
in a given experiment mean for human safety? How will expression responses in rats be correlated with those in dogs, monkeys or humans? How do certain transcript changes statistically correlate with chemical dose or with traditional endpoints such as clinical chemistry or histopathology. How do pathways and genes compare across species? Finally, does a given probe actually measure a given activity, a splice variant, or even closely related sequences? These questions are in the language of toxicology, but require the tools of bioinformatics, where the sequence and computer rule, and while NDR1 and CAP43 ARE the same gene, c-src and Src ARE not. Toxicologists unfamiliar with these tools and concepts will be a great disadvantage in the coming decades, and the challenge of training programs is to incorporate this knowledge for both faculty and students alike.

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

D. L. Eaton, Dept. of Environmental Health, University of Washington, Seattle, WA.

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

DISTINCT ISOFORMS OF CALCIUM-INDEPENDENT PHOSPHOLIPASE A, (iPLA), ARE RESPONSIBLE FOR APOPTOSIS OF ENDO THELIAL CELLS INDUCED BY 1-METHYLANTHRACENE (1-MA) AND PHENANTHRENE (PA).

P. K. Tithof, M. Elguazzar, K. P. Lu and K. Ramos, University of Tennessee, Knoxville, TN and Texas A&M University, College Station, TX.

Smokers demonstrate a significant increase in endothelial cell (EC) injury when compared to non-smokers, a response associated with apoptosis. Specific components of cigarette smoke that induce apoptosis have not been identified. 1-MA and PA are polycyclic aromatic hydrocarbons present in high concentrations in cigarette smoke. This study was conducted to test the hypothesis that 1-MA and PA induce apoptosis of cultured human coronary artery ECs (HCAECs). HCAECs were grown to 90% confluence and treated with 1-MA, 100 μM, PA or vehicle for various times. Apoptosis was detected by confocal microscopy of Annexin V/Propidium iodide staining and by Western analysis for cleavage of poly(ADP)ribosepolymerase.

Apoptosis was evident after 1 hour of treatment with 1-MA or PA, but not vehicle. Given that apoptosis of many cell types involves iPLA, activity, subsequent experiments were conducted to evaluate the role of iPLA, in EC apoptosis. ECs were labeled for 24 hr with 0.25 μCi/ml [3H]arachidonic acid (AA) and release of [3H]-AA into the medium was used as a measure of iPLA, activity. Gene expression for various iPLA, isoforms was evaluated by reverse transcription-polymerase chain reaction (RT-PCR).

Both 1-MA and PA induced apoptosis, and time-dependent release of [3H]-AA that preceded the onset of apoptosis. Furthermore, the inhibitor of Group V iPLA, berenil and bile, inhibited [3H]-AA release and apoptosis induced by 1-MA, but not PA. Conversely, the inhibitor of acidic iPLA, Mj33, significantly attenuated [3H]-AA release and apoptosis in response to PA, but not 1-MA. RT-PCR experiments demonstrated that the genes for these two enzymes are expressed in HCAECs. Collectively these data suggest that 1-MA and PA induce apoptosis by activating distinct isoforms of iPLA, This effect may be relevant for the mechanism by which cigarette smoking causes EC injury. (Supported by American Heart Association Grant #016264B).

STATIN INDUCED APOPTOSIS IN DIFFERENTIATED RAT SKELETAL MUSCULAR CELLS.

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

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

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

Y. Do, S. Ryu, M. Nagarkatti and P. S. Nagarkatti, Microbiology and Immunology, Medical College of Virginia, Richmond, VA and Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA.

Previous studies from our laboratory have demonstrated that β-estradiol-17β-valorate (E2) induces apoptosis in thymocytes leading to thymic atrophy. In the current study we investigated the role of Fas-FasL interactions in E2-induced thymic atrophy and susceptibility to apoptosis using C57BL/6 wild-type (+/-), Fas-deficient (C57BL/6-ko/lpr/lpr and Fas-deficient (C57BL/6 gld/gld) mice. The +/-, lpr and gld mice were treated with various concentration of E2 such as 75, 25, 5, 1, or 0.1 mg/kg body weight or vehicle. The thymic from these mice were harvested on days 1, 4, and 7 following treatment and cellularity and apoptosis were determined. Treatment with E2 caused a decrease in thymic cellularity at all doses except 0.1 mg/kg, in all three groups of mice, particularly on days 4 and 7. Interestingly, however, the degree of thymic atrophy in lpr and gld mice was significantly less than that seen in wild-type mice. When thymocytes were analyzed for apoptosis, cells from lpr and gld mice showed decreased levels of apoptosis. Moreover, cDNA array analysis of gene expression revealed that treatment with E2 upregulated several genes involved in apoptosis including Fas, caspases, and TRAIL. At higher doses, E2 caused a decrease in cellularity of the spleen in wild-type mice. Also, E2 treatment decreased the proliferative response of T cell to the superman, SEB, and when Fas-FasL expression was determined, to be not significantly expressed on activated T cells by inducing Fas, gene and triggering apoptosis through paracrine or autocrine pathways (supported in part by NIH grants: ES 09098, HL 58641 and AI 01392).

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

N. C. Brown, M. Nagarkatti and P. Nagarkatti, Microbiology and Immunology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA and Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA.

Endocrine disrupting compounds like estrogens have the ability to alter the immune system. One such compound is a synthetic nonsteroidal estrogen, diethylstilbestrol (DES). Studies from our Lab. have demonstrated that estrogen induces
rhymic atrophy by triggering apoptosis. In the current study, we investigated the ability of DES to induce apoptosis in vitro in murine lymphomas, LSA and EL-4 as well as human lymphomas and leukemias, Jurkat, Sup T1, HUT 78 and Molt-4. For this purpose, tumor cells were cultured with DES (50, 25, 10, 1 microM) in serum-free medium. Twenty four hours later, the cell numbers were enumerated by trypan blue dye exclusion and apoptosis was detected by TUNEL staining. Studies demonstrated that DES at concentrations of 10-50 microM induced varying levels of apoptosis in all the cell lines tested. In contrast, as Fas-Fas ligand (FasL) interaction plays a crucial role in induction of apoptosis, further studies were performed to assess the level of expression of these molecules on the cells treated with DES. It was observed that while Fas expression was not altered, there was significant upregulation of FasL. The induction of apoptosis by DES was inhibited by treatment with caspase inhibitors including z-VAD-fmk, z-WEHD-fmk, z-VAD-0-mk and z-IETD-fmk. Together, these studies demonstrated that DES-induced apoptosis of tumor cells may involve the death receptor pathway (Supported in part by grants from NIH: ES09098 and HL58641).

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


Microbiology and Immunology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA, and Pharmacology and Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA.

Exposure to delta 9-tetrahydrocannabinol (THC), an active component of marihuana can lead to suppression of the immune response although the mechanism remains unclear. In the current study, we tested the hypothesis that cannabinoids induce toxicity in lymphoid cells by triggering apoptosis. To this end, we screened a large number of murine and human transformed cells of immune origin for the expression of cannabinoid receptors and their susceptibility to apoptosis induced by THC and other cannabinoid receptor agonists. RT-PCR and Western blot analysis revealed that murine tumor cell lines EL-4, LSA, and P815 expressed both the CB1 and CB2 receptors. Exposure of EL-4, LSA, and P815 cells to THC led to a significant reduction in cell viability and increase in apoptosis. Exposure of EL-4 tumor cells to the synthetic cannabinoid, HU-210 also led to significant induction of apoptosis, while exposure to WIN55212 was less effective. Treatment with THC in vitro led to a significant reduction in the number of viable tumor cells and an increase in tumor cell apoptosis. Examination of a number of human leukemias and lymphomas revealed that unlike the murine tumors, Jurkat, Molt-4, and Sup-T1 cells express only CB2 receptors. Exposure of these tumor cells to 5 uM THC or HU-210 led to significant induction of apoptosis. The induction of apoptosis by THC in tumor cells was mediated through the cannabinoid receptors trasmetham as it was inhibited in the presence of CB1 and/or CB2 receptor antagonists, SR14179 and SR145528. Together these data demonstrate that ligation of cannabinoid receptors can induce apoptosis in transformed immune cells. Thus, cannabinoids may induce immunotoxity by triggering apoptosis in lymphoid cells through cannabinoid receptors. These studies also suggest that cannabinoid receptor agonists may serve as anticancer agents (Supported in part by grants NIH DA14885, ES09098 and HL58641).

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

S. H. I. Hassan and D. Ross.

Department of Biomedical Science, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia and Department of Pharmaceutical Sciences, University of Denver, CO.

Hydroquinone (HQ), a benzene metabolite induces apoptosis in hematopoietic cells and is believed to be involved in the pathophysiology of benzene-induced aplastic anemia. Here, we investigated the involvement of mitochondria and activation of the apical caspase-9 in HQ induced apoptosis in myeloperoxidase (MPO)-rich HL-60 and MPO-deficient Jurkat T cell. Treatment of HL-60 and Jurkat cells with HQ (25uM and 50uM respectively) resulted in comparable apoptosis as assessed by flow cytometry and biochemical changes, including phosphatidyllylycerol (PS) exposure, loss of mitochondrial membrane potential (MMP) and caspase-9 cleavage. In HL-60 cells, pretreatment with the pan-caspase inhibitor benzoyloxycarbonyl-Val-Ala-Asp fluoromethylketone (ZVAD) did not inhibit PS exposure and the loss of MMP was relatively unaffected. Interestingly, activation of caspase-9 was only inhibited by the P38/5kDa subunits without any intact protein observed. In marked contrast, HQ-treated Jurkat cells, ZVAD significantly abrogated PS exposure and loss of MMP with caspase-9 remained in its zymogen form. These data suggest that in HQ-treated HL-60 cells, the event(s) leading to mitochondrial alteration occurs via a ZVAD insensitive mechanism suggesting for a caspase-independent pathway in HQ treated HL-60 cells. In contrast, in HQ-treated Jurkat cells, the event(s) leading to the participation of mitochondrial/caspase-9 occurs in a ZVAD sensitive manner indicating a caspase-dependent process in this apoptotic death. These differential effects may be attributed to MPO-catalyzed activation of HQ to highly reactive quinones and reactive oxygen species in HL-60 cells and, as a result, specific death pathway is recruited. (Supported in part by ES 09554)

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

T. Qian and J. L. Lemasters.

Cell & Developmental Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Onset of the mitochondrial permeability transition (MPT) is a key event signaling TNF-α-induced apoptosis in cultured rat hepatocytes. Previously, we showed that pre-exposure to non-toxic oxidative stress sensitizes hepatocytes to the TNF-α-induced M2T and apoptosis. In the current study, we tested whether exposure of cytosolic and mitochondrial superoxide dismutase (SOD1 and SOD2, respectively) inhibits the MPT and apoptosis promoted by TNF-α plus oxidative stress. Rat hepatocytes were incubated with an adenosine (AdS5K, 30pM) for 2 hours to induce PB superpressor. Some hepatocytes were also incubated with adenosines (30 pM) expressing SOD1 or SOD2 for 3 hours. Afterwards, the hepatocytes were exposed to 1 μM paracetamol (PAPA) and mitochondrial ROS generation, mitochondrial membrane potential (TMMP) and mitochondrial membrane permeability were measured by confocal microscopy. In t-BuOOH pretreated cells, TNF-α activated ROS formation, as indicated by a 38% increase of DCF fluorescence over 3 h. In hepatocytes expressing SOD1, ROS was inhibited to 182%. SOD2 also inhibited TNF-α-induced cell killing from 68% after 12 h to 25%. Expression of SOD1 did not protect against cell killing. Confocal microscopy data and measurement of mitochondrial ROS generation, mitochondrial membrane depolarization and mitochondrial membrane permeabilization, which began 4 h after TNF-α plus t-BuOOH. SOD2 expression suppressed mitochondrial ROS, depolarization and permeabilization even after 8 h. In conclusion, mitochondrial superoxide generation sensitizes cultured rat hepatocytes to TNF-α-induced apoptosis by promoting onset of the MPT. Expression of mitochondrial but not cytosolic SOD blocks this apoptotic signaling.

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


Biochemistry and Molecular Genetics, CIT Center for Health Research, Research Triangle Park, NC. Sponsor: J. Corton.

Peroxisome proliferators (PP) are a large class of structurally dissimilar chemicals that induce hepatocellular proliferation and peroxisome proliferation through the nuclear receptor family member peroxisome proliferator-activated receptor alpha (PPARα). We performed gene expression profiling to examine differences in gene expression between untreated wild-type and PPARα-null mice. PPARα-null mice exhibited altered expression of a number of genes that play roles in apoptosis, favoring decreased susceptibility to apoptosis. We hypothesized that PPARα activation positively influences induction of apoptosis in the liver. Wild-type and PPARα-null mice were treated in separate experiments with two apoptosis inducers: J2-antibody, which induces the Fas-dependent death pathway, and concanavalin A (ConA), which induces apoptosis through an unknown pathway. Mice were treated with and without the PP, WT-14, 0.5 (WT), Wild-type mice treated with WT were more sensitive to induction of apoptosis by J2 antibody than untreated wild-type mice or untreated or WT-treated PPARα-null mice. After exposure to J2, morbidity and mortality were significantly higher in the presence of WT in wild-type mice and protected by PPARα-null mice. Wild-type mice treated with WT were also more sensitive to the apoptotic effects of ConA. We examined the expression of a number of gene products in the liver that influence apoptosis including those found to be altered by gene expression profiling. In the absence of apoptosis-inducer treatment, wild-type mice treated with WT exhibited increases in the activated form of caspase-9 which paralleled the decrease in the anti-apoptotic protein, Bcl-2. A member of the Bcl-2 family. These data demonstrate that PPARα activation can significantly enhance the sensitivity of apoptosis inducers in the mouse liver possibly through alteration in the expression of proteins that regulate apoptosis.
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S(1,2-DICHLOROVINYL)-L-CYSTEINE (DCVC)-INDUCED CHANGES IN EXPRESSION OF GENES RELATED TO APOPTOSIS AND CELL GROWTH IN HUMAN PROXIMAL TUBULAR CELLS.


DCVC, the cysteine conjugate of trichloroethylene, is a potent nephrotoxicant. Using a broad range of concentrations, including environmentally relevant ones (10 to 500 µM), we showed that DCVC induces necrosis, apoptosis and cell proliferation in a time- and concentration-dependent manner in primary cultures of human proximal tubular (hPT) cells. Significant apoptosis was observed with as low as 10 µM DCVC at 1 h, using cell cycle analysis with propidium iodide staining or annexin V staining and flow cytometry. Maximal apoptosis occurred with 50 µM DCVC at 4 h incubation. Similar time course was observed for enhanced cell proliferation, as assessed by cell cycle analysis with flow cytometry and incorporation of radiolabeled thymidine. In contrast, significant increases in necrosis (as assessed qualitatively by morphology and quantitatively by release of lactate dehydrogenase) did not occur until later incubation times (24 hr) and higher DCVC concentrations (≥100-200 µM). To gain more insight into the regulation of these responses, we measured protein expression of key regulatory molecules that are related to apoptosis and cell growth regulation. Primary cultures of hPT cells were incubated with 10, 50, 100, 200, or 500 µM DCVC for 0.5, 1, 2, 4, 8, or 24 hr and protein expression was determined by Western blot analysis. Expression of several proteins related to apoptosis were altered, including Bax (decrease), Apaf1, Bcl2 and caspase-9 (all increase), and cSrc (increase in low molecular weight fragments). C-Jun increased only at low DCVC concentrations, whereas expression of Hsp27, Nrf2, p53 subunit, and p53 all showed modest to large increases that were clearly dependent on DCVC concentration and exposure time. These results are consistent with the prominence of apoptosis at low concentrations of DCVC and with a role for oxidative stress and changes in regulation of cell growth in the response of hPT cells that are exposed to DCVC. (Supported by NIEHS Grant ES08828.)

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

A. L. Nieminen, L. Li, G. Mo and F. Agani. Department of Anatomy, Case Western Reserve University, Cleveland, OH.

Tumor hypoxia develops in most solid tumors because of inadequate vasculature or abnormal vascular architecture. Frequently, these hypoxic cancer cells resist radiation and chemotherapy. The aim of this study was to characterize the roles of p53 and the Ras/Rap1b inactivation pathways in hypoxia-induced apoptosis. p53+/− and p53−/− mouse embryonic fibroblasts (MEF) transformed with proto-oncogenes Ras and EIA were exposed to hypoxia for 7 h. Hypoxia caused 29 ± 4.2% and 4 ± 1.2% apoptosis in p53+/− and −/− MEF, respectively, as assessed by changes in nuclear morphology. Hypoxia had no effect on p53+/+ and −/− MEF without Ras and EIA. Interestingly, the specific inhibitor of mitogen-activated protein kinase (MKK1), PD98059 (50 µM), accelerated hypoxia-mediated apoptosis (85 ± 6.6%) in p53+/+ but not p53−/− MEF. PD98059 alone caused 45 ± 8.8% apoptosis in p53+/+ MEF. To investigate whether apoptosis induced by inhibition of MKK1 was mediated by activation of p38 MAPK pathway, we measured caspase-8 activation by flow cytometric assay and Western blot. Both methods showed that exposure to hypoxia or PD98059 alone did not activate caspase-8. However, the combination of hypoxia and PD98059 caused a 2-fold increase of caspase-8 activity in p53+/+ MEF but not in p53−/− MEF. To determine whether p38 MAPK activation caused translocation of Bax from the cytosol to mitochondria, we transfection p38 MAPK MEF with cDNA Bax-GFP. Before hypoxia, the pattern of Bid-GFP fluorescence was diffuse indicating a cytosolic localization. After hypoxia Bid-GFP fluorescence became punctate in the presence of PD98059 and colocalized with Mitotracker Red. Collectively, our results indicate that p38 MAPK pathway inhibits hypoxia-induced apoptosis, and we conclude that the Ras/MAPK pathway provides a survival signal against p53-mediated hypoxia apoptosis. MAPK inhibitors may therefore be useful as an adjunct chemotherapy for hypoxic solid tumors. Supported by NS59469.

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

J. Yao, F. Pisha, X. Liu, Y. Li, L. Yu and J. L. Bolton. Department of Medicinal Chemistry and Pharmaceutics, University of Illinois at Chicago, Chicago, IL.

Excessive exposure to synthetic and endogenous estrogens has been associated with the development of cancer in several tissues including the breast. 4-Hydroxyquinolin (4-OHEN), a major metabolite of equine estrogens present in Premarin® estrogen replacement formulation, has been shown to induce DNA damage and apoptosis in human breast cancer cells. It also has the potential to be a tumor promoter and complete carcinogen in vitro. To further understand the mechanism(s) of estrogen carcinogenesis, we have used human non-tumorigenic breast epithelial MCF-10A cells to study the toxic effects of 4-OHEN. We observed cytotoxicity in MCF-10A cells induced by 4-OHEN. LC50 for 18 h-treatment was 10.7 ± 1.9 µM. 4-OHEN also caused dose-dependent increases in apoptosis and DNA damage as measured by the DAPI nuclear staining assay and the Comet assay, respectively. In the present study, we found that treatment with 100 nM 4-OHEN biweekly for 2 weeks induced a 50% increase in these human breast epithelial cells. Interestingly, after the treatment of cells with 100 nM 4-OHEN biweekly for 4 weeks, the treated MCF-10A cells were resistant to 4-OHEN- or cisplatin-induced apoptosis. The mechanism of cellular resistance to apoptosis is currently under investigation. Our data indicated that long-term low-level estrogen exposure could induce DNA damage and initiate cells that become resistant to apoptosis, which could contribute to the mechanism(s) of equine estrogen carcinogenesis. (Supported by NIH grant CA73638.)

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

D. C. Jones, S. S. Lau and T. J. Monks. Center for Molecular & Cellular Toxicology, University of Texas at Austin, Austin, TX.

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

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

P. G. Forkert, L. H. Lash, V. Nadeau and R. Tardif. Anatomy and Cell Biology, Queen's University, Kingston, ON, Canada, Pharmacology, Wayne State University, Detroit, MI and Environmental & Occupational Health, University of Montreal, Montreal, PQ, Canada.

We have tested the hypothesis that TCE is metabolized by CYP2E1 and elicits toxicity in the male reproductive system. We have exposed mice to TCE by inhalation (1000 ppm) for 6 h per day for 5 days per week for a total of 1 to 4 weeks. Internal exposure to TCE was monitored by measuring urinary levels of the TCE metabolites, trichloroacetic acid (TCA) and trichloroethanol (TCHO). We have determined CYP2E1 expression in epididymis and testis by i) immunohistochemical staining, ii) immunohistochemical staining, and iii) measurement of p-nitrophenol hydroxylase activity. We have also determined formation of chloral in incubations of microsomes from epididymis and testis and in microsomes preincubated with a CYP2E1 antibody. Our results showed that urinary levels of TCA and TCHO increased between the first and second week, and remained elevated thereafter. CYP2E1 is localized in epididymal cells of the epididymis and Leydig cells of the testes. Immunoblot of CYP2E1 showed that the protein was present at higher levels in epididymis than in testis. This finding coincided with the 2-fold higher level of hydroxylase activity detected in epididymis vs. testis. Formation of chloral in incubations of microsomes from epididymis and testis was time- and concentration-dependent. Peak levels were detected at 60 min of incubation time at a concentration
FORMATION OF FREE RADICALS FROM METABOLISM OF 1-DICHLOROETHYLENE (DCE) IN MURINE AND FISH LIVER AND MICROVITAMINS, M. Monbouquet, T. Bray, E. Ho, T. Boulac, C. R. Hille, G. Herni, M. Collins, and P. F. Fod. Chemistry, Queens University, Kingston, ON, Canada. 

Human Nutrition, Ohio State University, Columbus, OH, and Molecular & Cellular Biochemistry, Ohio State University, Columbus, OH.

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

RELATIONSHIP OF THE SINGLE-ELECTRON REDUCTION POTENTIAL OF QUINONES TO THEIR ABILITY TO UNDERGO REDOX CYCLING MEDIATED BY RAINBOW TROUT MICROSMAL FLAVOPROTEINS S. Carney, J. Serrano, P. K. Schmieder and L. R. Henry, Biotechnology Training Program, University of Wisconsin, Madison, WI, and Mid-Continent Ecology Division, USEPA, NHIEEL, Duluth, MN and Experimental Taxonomy Division, USEPA, NHIEEL, Duluth, MN.

Exposure to quinones can result in cytotoxicity due to electrophilic arylation or redox cycling via enzymatic reduction to the semiquinone radical with subsequent transfer of an electron to molecular oxygen to form superoxide anion radical (O_2^-). Subsequent regeneration of the parent quinone. To assess the relationship between the redox cycling ability of quinones in rainbow trout and their single-electron reduction potentials (E), the reduction of O_2^- by a series of quinones with potentials ranging from -348 to +671 mV was determined using two assays. Rainbow trout liver microsomes, containing NADPH cytochrome P450 reductase and NADPH, were exposed to quinones in the presence of NADPH or NAPDH. Generation of O_2^- was measured as O_2 consumption and acetylated cytochrome c reduction. The reduction of acetylated cytochrome c is a more sensitive indicator of O_2^- production. The peak and lower limit for acetylated cytochrome c reduction were E = 0.96 mV and -0.84 mV with NAPDH cofactor and E = 0.36 mV and -0.36 mV with NADPH cofactor. A narrower range of redox activity was observed with the O_2 consumption assay. The peak rate for oxygen consumption was observed at the same E for the acetylated cytochrome c assay and the lower limit for reduction was E = -0.34 mV with both NADPH and NAPDH. These redox windows for rainbow trout microsomes are comparable to those reported for NADPH cytochrome P450 reductase and NADPH-cytochrome c. Redox properties of quinones in rainbow trout were tested in rat hepatocytes. In both rats and rainbow trout the peak reduction occurs at a more positive E with NADPH cofactor as compared to that with NADPH cofactor. In contrast to rat flavoproteins, the peak rate of quinone redox cycling facilitated by rainbow trout flavoproteins occurred with NADPH as the cofactor rather than NADPH. This abstract does not necessarily reflect EPA policy.
functional p53 allele was a common feature of the benzene-induced tumors supporting the use of these mice to examine the molecular mechanisms of cancer. We then examined the benzene-induced tumors for altered expression of genes involved in the retinoblastoma (Rb) pathway since this pathway is also commonly altered in human tumorigenesis. Real-time quantitative RT-PCR and Western blot analysis were used to determine the levels of genes involved in the Rb pathway (p15INK4b, p16INK4a, p19ARF, Rb, cyclin D1, cyclin D2, and E2F-1) in 18 benzene-induced thymic lymphomas from p53+/- mice. Virtually all genes showed decreased mRNA levels in the tumors, however all tumors showed increased levels of p16INK4a mRNA and protein compared to normal thymus, suggesting that increased levels of p16INK4a mRNA can be used as a biomarker for benzene-induced transformation. Bisulfite DNA sequencing showed that the tumors exhibited reduced methylation at specific CpG sites in the promoter region of p16INK4a suggesting that altered methylation contributed to the p16INK4a mRNA increases. Our data show that benzene-induced thymic lymphomas from p53+/- mice exhibited alterations common to certain human cancers, providing further support for the use of these mice as a model to examine the mechanisms involved in human carcinogenesis.

299 PROGRESSIVE ALTERATIONS IN DNA METHYLATION DURING TUMORIGENESIS.


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

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

S. Yang, L. Li, W. Wang, Y. Li, M. Edelbrock, P. M. Kramer, M. A. Pereira and L. Tao. Pathology, Medical College of Ohio, Toledo, OH.

Dichloroacetic acid (DCA), trichloroacetic acid (TCA), dibromoacetic acid (DBA), bromochloromethane (BCDM), methylene chloride (MC) and arsenic (As) are non-genotoxic carcinogens in various organs. DNA methylation as 5-methylcytosine (5-MeC) is a mechanism to regulate the transcription of mRNA. Hypomethylation of DNA and of genes including insulin-like growth factor 2 (IGF-II) and the c-myc protooncogene has been proposed to be an epigenetic mechanism for non-genotoxic carcinogens. We determined the effect of DCA, TCA, DBA, BCDM, MC and As on methylation in the promoter of the c-myc gene and in the differential methylation region 2 of the IGF-II gene. B6C3F1 mice were administered in their drinking water 3.2 mg/L DCA or 4.0 mg/L TCA for 24-36 hours and for 28 days or were administered DBA (1000 and 2000 mg/kg) and BCDM (350 and 700 mg/kg) for 2-28 days. Methylation mechanisms in mice by inhalation of 1.0, 4.4, and 10.4 mg/m3 for 5 days. Mice received by gavage dimethylaminoacetic acid (DMA) at 600 mg/kg for 5 days. Fisher 344 rats were administered for 2-28 days, DBA (1000 or 2000 mg/kg) and BCDM (350 or 700 mg/kg) in drinking water or were administered BCDM (50 and 100 mg/kg) by gavage. Methylation of the two genes was determined using the bisulfite-modified DNA sequencing procedure. Methylation in the IGF-II and c-myc genes was decreased in the liver and kidney of BCDM, DMA and MC-treated mice, in the kidney of BDCM and DMA-treated mice, in the skin of DMA-treated mice, in the bladder with DCA, TCA and DBA-treated mice, in the lung of MC-treated mice and in the liver of BCDM and DBA-treated rats. Hypomethylation of the genes was observed after exposure of at least 96 hours and was maintained for 28 days. These findings suggested that hypomethylation of DNA and of genes is an epigenetic activity of non-genotoxic carcinogens so that it could be involved in their mechanism.

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

L. Tao, Y. Li, S. Yang, W. Wang, P. M. Kramer and M. A. Pereira. Pathology, Medical College of Ohio, Toledo, OH.

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

302 EXPRESSION OF THE ARL4 HYDROCARBON (AH) RECEPTOR AS A MARKER OF HUMAN BREAST CANCER PROGRESSION.

X. Yuan*, H. M. Gao*, C. R. Jeffers* and S. E. Elton*. Pharmacology, Meharry Medical College, Nashville, TN and Pharmacology, University of Wisconsin, Madison, WI.

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

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


Gap junction intercellular communication (GJC) is thought to play an important role in maintaining tissue homeostasis by influencing cell growth and cell death. Disruption of GJIC via down-regulation of connexin (Cx) proteins has been sug-
gested as a potential mechanism involved in the action of many non-genotoxic carcinogens. We have investigated the time- and dose-dependent effects of non-genotoxic carcinogens on connective tissue expression and cell proliferation in target and non-target tissues with TCDD (0.0025, 0.025, 0.25 µg/kg), Wy-14, 643 (50 µg/kg), chloroform (18, 90, 180 µg/kg) and p-dichlorobenzene (DCB) (30, 150, 300 µg/kg) for 3 and 28d. Tissue sections were immunolabelled for Casp2, CD2 and PCNA. Treatment with Wy-14, 643 led to a significant increase in PCNA-labelled hepatocytes (indicative of proliferation) and reduction in the number of abnormal area of Casp2-labelled plaques, quantified in random regions. Similarly, administration of TCDD resulted in decreased Cx expression in both liver and thyroid, but without changes in cell replication. No effects were seen in kidneys (non-target organ) of TCDD and Wy-14, 643 treated animals. In contrast, a dose-dependent reduction in Casp2 was detected in control rats treated with the male rat kidney carcinogens chloroform and DCB at both time points, while changes in proliferation were seen only following 3d treatment with 180 µg/kg chloroform and 28d treatment with 300 µg/kg DCB. In the liver (not target organ), chloroform and DCB led to a transient increase in proliferation without effects on Cx expression. In summary, modulation of Cx expression in liver was observed in target organs following short-term treatment with non-genotoxic carcinogens. No changes were seen in non-target organs analysed. While aberrant expression of Cx plaques did not correlate with proliferative response to carcinogen treatment, the interaction of both processes may be important in non-genotoxic carcinogenesis and determination of threshold effect levels.

304 QUANTITATIVE PROTEOMIC ANALYSIS OF MOUSE LIVER RESPONSE TO THE PEROXISOME PROLIFERATOR DIETHYLPHTHALATE (DEHP).
N. Macdonald{
S. Chevalier{
T. Inoue{
M. Davison{
R. Rowlinson{
J. Young{
S. Rayner{
R. A. Roberts{
HAES Research, Singapore CIL, Masschedef, United Kingdom{
Pfizer, Discovery Support, Ambiose, France and Astrazeneca, Pharmaceuticals, Masschedef, United Kingdom.

Peroxi"som e proliferators (PPs) are a diverse group of chemicals that cause hepatic proliferation, suppression of apoptosis, peroxisome proliferation and liver tumours in rodents. The biochemical responses to PPs involves changes in the expression of peroxisomal oxidation enzymes and fatty acid transport proteins such as acyl CoA oxidase (ACO) and liver fatty acid binding protein (L-FABP). The response to PPs is mediated by the peroxisome proliferator activated receptor α (PPARα) and the ligands of PPARα non transgenic mice do not develop tumours in response to PPs. In order to identify the molecular pathways underlying the adverse effects of PPs in rat tissues, rats were treated 2d peroxisome proliferator-activated receptor α (2D-DIGE) to provide quantitative proteomic analyses of diethyphthalate (DEHP) treated wild type or PPARα null mouse livers. Since tumourigenesis is both PP and PPARα dependent, analyses were focused on these changes. Fifty nine proteins were identified where altered expression was both PPARα and PPARα dependent. In addition, 6 proteins regulated by the deletion of PPARα were identified possibly indicating an adaptive change in response to the loss of this receptor. The proteins identified as being regulated by PPARα are known to be involved in lipid metabolism pathways, but also in amino acid and carbohydrate metabolism, mitochondrial bioenergetics and the immune system. The changes have been reported to be regulated by PPARα. These data provide novel insights into the pathways utilized by PPs and may assist in the identification of early markers related nongenotoxic hepatocarcinogenesis.

305 CHARACTERIZING THE MOUSE LIVER TUMOR RESPONSE THROUGH EXPRESSION PROFILE ANALYSIS.
P.D. Cornwell{
S. E. Anderson{
G. V. Bobashev{
J. L. Cordon{
CITR Centers for Health Research, Research Triangle Park, NC{
GlassSmithKnight, Research Triangle Park, NC{
Research Triangle Institute, Research Triangle Park, NC.

Human carcinogen risk is commonly assessed in the B6C3F1 mouse strain, a cross between a hepatocarcinogen-resistant (C57BL/6) and a hepatocarcinogenic-sensitive (C3H/He) strain. Other mouse strains are known to vary in their hepatocarcinogenic sensitivity as well. Although a number of hepatocarcinogenic sensitivity/resistance (hep/cr) loci have been mapped, the molecular mechanisms for these sensitivity/resistance are unknown. We hypothesize that expression differences of key genes in these loci are responsible for some of the observed strain variation. Hepatic transcript profiles of 7 mouse strains (AJ, AKR/J, B6C3F1, C3H/HeJ, C57BL/6J, C57BB/6j, DBA/2J) and profiles of chemically (DEN, Wy-14, 643) induced hepatic adenomas were obtained using the Atlas Mouse Cancer 1.2 array (Clontech) The Phenotypic Trait Value (PTV) method, which correlates phenotypic data with gene expression levels, was used to analyze these data. Identified in the analysis were 144 genes related to hepatocarcinogenic sensitivity, 45 genes related to the rate of appearance of hepatic foci (conversion rate), 138 genes related to the rate of increase in hepatic foci size (propagation rate), 264 genes related to DEN-induced adenomas and 144 genes related to Wy-14, 643-induced adenomas. Of these genes, 78 are located near hep/cr loci and may play a critical role in differential tumor susceptibility. In addition, many of these genes fall into classes of genes related to malignant transformation (e.g., cell cycle regulators). In summary, we used multiple data sources to focus on genes whose expression most clearly explains the differences between resistant and susceptible mouse strains. These differences determine sensitivity to a chemical carcinogen will lead to a better understanding of the mechanisms of hepatocarcinogenesis in mice and a better understanding of the relevance of the mouse liver tumor response in human risk assessments.

306 ACYCLONITRILE EFFECTS ON GENE EXPRESSION IN RAT BRAIN AND GLIAL CELLS.
Z. Jian{
L. M. Kamendulis{
Y. Xu{
J. F. Kapins{
Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

Chronic exposure to acrylonitrile induces astrocytomas in rats. While the mechanisms for acrylonitrile-induced carcinogenicity are not completely understood, previous studies have shown an induction of oxidative stress in acrylonitrile treated rat brain and rat glial cells. In the present study, we investigated gene expression changes in rat brain following treatment with 200 mg/kg acrylonitrile for 14, 28, and 90 days and in glial cells treated with 25, 50 or 75 mg/ml acrylonitrile for 24 hours. Using cDNA array technology, an up-regulation in several genes associated with cell growth regulation (c-myc, c-jun, er-1, p27kip2, IL-2 and gadd45a protein) were observed in rat brain following treatment with acrylonitrile for 14 days. After 28 and 90 days of treatment with acrylonitrile, expression of these genes was lower than that seen at 14 days and gene expression of c-fos and c-jun was lower than control. An elevation in NF-KB pathway was seen at all time points. In acrylonitrile-treated glial cells of rats exposed to 25 mg/ml acrylonitrile, NF-KB signal ning were up-regulated while gene regulation of cell proliferation pathways were down-modulated and genes involved in the apoptotic pathway were up-regulated at 24 hours. Since NF-KB activation is regulated post-transcriptionally, by translocation of the transcription factor from the cytoplasm to nucleus, a minor change in mRNA proteins may represent a significant usage (or activation) of NF-KB early after acrylonitrile treatment. Activation of NF-KB by acrylonitrile was therefore confirmed in glial cells with a reporter gene assay utilizing glial cells transfected with an NF-KB plasmid. The results provide important information on the genes modulated in the brain by treatment with acrylonitrile. Since many of the observed modulated genes are regulated by reactive oxygen species, it is likely that these changes in gene expression are due to acrylonitrile-induced oxidative stress.

307 ALTERED GENE EXPRESSION ASSOCIATED WITH FURAN INDUCED CHOLANGIOFIBROSIS IN THE RAT.
J. M. Hitchcock{
K. C. Hickling{
V. Orefio{
J. K. Chipman{
R. Coleman{
C. Kind{
J. Evans{
T. G. Hammond{
School of Biosciences, The University of Birmingham, Birmingham, United Kingdom and Safety Assessment, Astrazeneca Res&D Charnwood, Loughborough, United Kingdom.

Cholangiocarcinoma (CC) is the most common primary hepatic malignancy in humans after hepatocellular carcinoma, comprising 15% of liver neoplasms. Human in CC is usually not considered to be malignant epithelial and associated cholangioblasts. We have studied gene expression changes to help characterize stages in the development of CC in the rat. Rats were dosed with furan at 35mg/kg daily for 3 months and also with 1 month 'off'-dose prior to necropsy to characterise persistent changes. Lesions with an elevated proliferating cell nuclear antigen (PCNA)-labelling index, and associated cell populations with increased wild type p53, phosphorylated c-jun and cdk-1 protein expression, were compared to controls of control rats using the Affymetrix Rat Genome U34A Array. 310 genes were over-expressed in both 3 and 3 +1 month livers compared to control. 105 genes showed a reduced expression compared to control. A number of these changes clearly reflect the shift in major cell type and fibrosis associated with the lesion. However a sub-population of genes with established roles in proliferation was altered in expression. These are currently being studied further using real time quantitative PCR (TaqMan) in conjunction with Laser Capture Microdissection (LCM) to establish these gene expression changes that distinguish the hyperplastic and metastatic regions from normal biliary epithelium.

308 MALIGNANT TRANSFORMATION BY MNNG AND ANTITRANSFORMATION BY ARSENIC OR METAL MIXTURE IN KERATOCYTES.
D. Bae{
J. S. Rhi{
R. J. Hana{
B. S. Yang{
J. A. Campani{
Environmental Health, Colorado State University, Fort Collins, CO, Center for Primate Disease Research, Rockville, MD and Anatomy & Neurobiology, Colorado State University, Fort Collins, CO.

In order to evaluate the carcinogenic potentials for chemicals such as arsenic (As) and other metals, we have identified molecular markers potentially involved in the process of carcinogenesis in keratocytes. We have demonstrated that treatment.
with MNNG enhanced transformation of the immortal human keratinocyte cell line, RHEK-1. In contrast, As alone or in mixtures of As, Cd, Cr, or Pb inhibited this process. Microarray analysis showed unique gene expression patterns in RHEK-1 cells treated with the chemicals/mixtures. To explore markers that may be involved in the enhancement or inhibition of malignant conversion, 9 genes commonly affected by the MNNG and As/mixture treatments were examined for expression in more detail by kinetic PCR. These genes were mitogen-activated protein kinase (MAPK) protein, phospho-ERK1/2 (P-ERK1/2), phospho-Akt (P-Akt), phospho-p70S6k (P-p70S6k), and phospho-ERK5 (P-ERK5). The expression of these genes was up-regulated by MNNG, As, and the mixture. These findings support the hypothesis that As/mixture treatments may enhance the malignant transformation of keratinocytes through the activation of MAPK and Akt pathways.

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

A. A. Khan1, R. W. Copple1, L. M. Geleta1, M. M. Schulter1, M. M. Drzenwicza1, and M. N. Hiltz1. Toxicology, Alberta Research Council, Vegreville, AB, Canada and Institute for Methods and Controls, National Research Council, Ottawa, ON, Canada.

In Alberta, Canada, some agricultural land sites are also used for petroleum production. This study was done to assess the temporal effects of Pembina Cardium crude oil (PCCO) in rats. Pair-wise groups of rats (6/group) were exposed to either PCCO or potable water (control) by gavage at 2.5 ml/kg on days 0, 7, 14, 21, 28 on day 0, and 7, 14, 21, 28 on day 7, 14, 21, 28. The selected tissues were analyzed for chemical parameters, and aliphatic hydrocarbons in the tissue were identified. The results showed that the levels of xenobiotic-metabolizing enzymes and aliphatic hydrocarbons increased significantly in the liver, kidney, and lung tissues of the rats exposed to PCCO.

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

R. W. Copple1, A. A. Khan1, L. Geleta1, M. M. Drzenwicza1, N. N. Naz1, and M. N. Hiltz1. Toxicology, Alberta Research Council, Vegreville, AB, Canada and Institute for Methods and Controls, National Research Council, Ottawa, ON, Canada.

Food safety is a high priority in Alberta, Canada. The landscape in Alberta is often shared by the petroleum and livestock industries providing opportunity for ruminants to be exposed to oilfield substances. The implications of petroleum ingestion on food safety do not appear to be known. Sheep (n=11) were administered 10 mL of Pembina cardium crude oil (PCCO) kg body weight or 10 mL of potable water on study days 0, 14, 28, and 42. Blood for clinicopathological examination was collected across study days. The sheep were necropsied on study day 45 and fat, brain, liver, kidney and skeletal muscle were collected for hydrocarbon analysis. The n-alkanes (C20 to C35) were extracted in each sample and the quantity and quality of the hydrocarbons were made by gas chromatography - mass spectrometry techniques. The total concentration of the C10 to C35 n-alkanes found in tissues were: fat 61.3±5.7 SE (ppm), perirenal fat 70.2±7.8 ppm, tailhead fat 60.1±6.7 ppm, bone marrow fat 41.4±5.0 ppm, liver 4.7±0.43 ppm, brain 0.6±0.08 ppm, and kidney 0.1±0.08 ppm. The C10 to C35 n-alkanes were below detection level (<0.1 ppm) in skeletal muscle. Clinicopathological observations include a leucocytosis primarily due to a neutrophilia. Leukemia was observed in the lungs during necropsy. This study showed that oral exposure of sheep to crude oil has potential of being a food safety issue.
A COMPARISON OF THE EFFECTS OF SHORT TERM ORAL EXPOSURE TO 2, 4, 6-TRINITROTOLUENE (TNT), 1, 3, 5-TRINITRO-1, 3, 5-TRIAZINE (RDX) AND CYCLOCTOTETRAMETHYLENE-TETRANITRAME (HMX) IN NORTHERN BOBWHITE QUAIL (COLINUS VIRGINIANUS).

R. M. Gogul1, C. T. Larsen1, M. S. Johnson1, D. Jones1 and S. D. Holladay2
1Biomedical Science, Virginia Tech, Blacksburg, VA; 2Health Promotion and Preventive Medicine, US Army, Aberdeen, MD. Sponsored: S. Holladay.

Potential risk to wildlife from exposure to the explosives, 2, 4, 6-trinitrotoluene (TNT), 1, 3, 5-trinitro-1, 3, 5-triazone (RDX) and cyclical octotetramethylene-tetranitramine (HMX) has been a concern throughout numerous military installations where it has been found in the soil. To date, limited data have been generated on the effects of oral exposure to TNT and no data have been generated for RDX and HMX in an avian species. Acute oral exposure and sub-acute dietary exposure to TNT, RDX and HMX was therefore evaluated in a species commonly found at military installations, the Northern Bobwhite (Colinus virginianus). Adult male and female quail (N = 17/sex/dose) were given acute oral doses of each of the compounds at 4782, 3188, 2125, 1417, 945, 630, 280, 187, 125 mg/kg and evaluated for 14 days. Acute lethal toxicity was observed at 2125 mg/kg with TNT for both genders. RDX doses of 280 mg/kg and 187 mg/kg were acute lethal doses in the male and female, respectively. Interestingly, HMX failed to induce an acute lethal dose during the initial AID study. Even in a repeated study with concentrations as high as 10, 750 mg/kg, acute lethal toxicity to HMX could not be determined. Blood analysis with HPLC showed a strong correlation between acute toxicity and quantity of compound found in the blood. This suggests that these compounds varied in their ability to be absorbed into bird’s blood stream. Additionally, fecal analysis of the compounds showed an inverse correlation to the severity of toxicity. Further, fasting status of the birds also seemed to impact the absorption. These data suggest that Northern Bobwhite appear to be less sensitive than mammals to acute oral exposures of TNT, RDX and HMX.

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


Perfluorooctane sulfonic acid (PFOS) has been demonstrated to accumulate in the liver and blood of exposed organisms. The potential for these detergent-like molecules to interfere with normal lipid/protein interactions in blood is of concern given the importance of many of these interactions, particularly those involving the transportation and action of hormones. Does binding to any compound that might modulate the endocrine system, PFOS binding to proteins was investigated. The ability of PFOS to displace a variety of steroid hormones from their specific binding protein sites in serum was measured. PFOS had only a weak capacity to displace the steroid hormones estrogens and testosterone from serum binding proteins. The effects on corticosterone remained unchanged at the least PFOS concentrations. Corticosterone displacement potency increased with increasing chain length and sulfonic acid compounds were more potent than those with a carboxylic acid functional group. The estimated PFOS concentrations to cause these effects were 320 ng/ml or less, equivalent to a serum concentration of greater than 160 pM. Using mass spectrometry and six enzyme binding assays, PFOS was demonstrated to bind strongly to bovine serum albumin (BSA). The combined results from all studies demonstrate that BSA binds only one or two PFOS molecules per protein molecule. This binding ratio is relatively low compared to the known 8-11 binding sites for other ligands such as fatty acids. Concentrations of PFOS required to saturate this binding site would be in excess of 50 to 100 ng/ml, indicating that PFOS would cause significant environmental concentrations of PFOS is unlikely that PFOS would cause significant displacement of hormones from their serum binding proteins in wildlife.

QUANTIFICATION OF ATRAZINE (ATRA) AND DIAMINOCLORETOXIRTRAZINE (DACT) IN RAB WILE BLOOD UTILIZING GC/MS/SIM.


Atrazine (ATRA) is a selective chloro-s-triazine herbicide for broadleaf and grassy weed control in corn, sorghum, sugarcane, and other crops. Dianisoclorotrazine (DACT) is the major urinary metabolite seen (64-67% of total) in vivo studies utilizing radioabeled ATRA administered orally. We are developing a suite of analytical methods to assist pharmacokinetic model development and teachings on how to assess human exposures via urine analysis, and to assess protein binding of these compounds in blood and hair (refer to Ruble et al. UTILIZATION OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL TO PREDICT DOSE-DEPENDENT GSH DEPLETION AFTER ATRAZINE TREATMENT. 41ST Annual SOT National Meeting poster session). In this work, we have developed methods to measure ATRA and DACT in blood. Rat whole blood was spiked with ATRA and DACT and cleaned up with solid phase extraction (SPE). The SPE eluate and standards were derivitized with methyl iodide and tetra-butyln hydroxide (TBAOH), yielding the di- methyl derivative of ATRA and the tetra-methyl derivative of DACT. Derivatization is not needed for ATRA but is necessary for DACT. We based quantification of DACT by co-chromatographing with a radioactive DACT standard. Samples and standards were analyzed with Gas Chromatography/Mass Spectrometry (GC/MS) in Specific Ion Monitoring (SIM) mode. Results showed excellent recoveries of ATRA and DACT with detection limits near 100 ppb. These methods have also been employed to determine one time course of generations of chloro-s-triazines in blood following dosing in rats. This work was supported by STAR grant R-828610-01-0 and HRCA grants U01/CCUB807121-10.

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

J. A. Stetsen and J. G. Houston. Environmental Laboratory. U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Toxicity of 2, 4, 6-trinitrotoluene (TNT) and three of its degradation products, 2, 4-diamino-6-nitrotoluene (DANT), 2-amino-4, 6-dinitrotoluene (2-ADNT), and 1, 3, 5-trinitrobenzene (TNB) was determined utilizing two aquatic invertebrates. Differential toxicity of the degradation products was observed in two organisms. Neatharsia arenicollis, a marine polychaete, and Hyalella azteca, a freshwater amphipod. Implications of the differential toxicity in these aquatic organisms derive specific challenges when conducting an ecological risk assessment. To gain a better understanding of the mechanisms responsible for the changes in toxicity, investigations were conducted through a series of toxicity experiments focusing on oxidative stress and metabolism by cytochrome P450. These mechanisms were studied by exposing the organisms to TNT in combination with a cytochrome P450 inducer and inhibitor and two antioxidants. Toxicity of TNT was increased in a time and concentration dependent manner following exposure to clotrimazole, suggesting inhibition of cytochrome P450 decreases the metabolism and elimination of TNT. BNF modified the toxicity of TNT in a U-shaped dose and time dependent manner. BNF at 0.125 mg/l significantly decreased the toxicity of TNT suggesting that increased cytochrome P450 activity increases the elimination of TNT. Acetobut and toluene mitigated the toxicity of TNT to H. azteca. Pre-exposure to acorbic acid (0.15, 0.44, 0.88, and 1.32 mg/ml) significantly decreased mortality in a 96-hr exposure to 5 mg/ml TNT. Pre-exposure to toluene decreased mortality in a U-shaped dose-dependent trend; mortality was the lowest at 0.125-ml/4 toluene and 3 mg/l TNT. Based on these data it is suggested that TNT elicits its toxicity through oxidative stress. These results demonstrate the sensitivity of aquatic invertebrates may be the result of changes in metabolic capacity (cytochrome P450 activity) and antioxidant capacity of the organism.
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EXPOSURE AND HEALTH EFFECTS OF INHALED FLORIDA RED TIDE BREVEOTOKSIN ON HUMANS DURING A RED TIDE EVENT:

D. Baden, W. Abraham, L. Backer, J. Benson, G. Bossart, S. Campbell, Y. S. Cheng, R. Clark, L. Fleming, D. Johnson, B. Kripkovich, J. Naar, R. Pierce and R. Weissman. *HABLAB, UNC Wilmington, Wilmington, NC, 1Mount Sinai Hospital, Miami Beach, FL, 2CDC, Atlanta, GA, 3Lovacote Respiratory Research Institute, Albuquerque, NM, 4Harbor Branch Oceanographic Institution, Fort Pierce, FL, 5Dept. of Health, Tallahassee, FL, 6NIEHS MPBS Center, Univ of Miami, Miami, FL, 7Marine Laboratory, Sarasota, FL, and 8Florida Poison Information, Miami, FL.

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

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PARALYTIC SHELLFISH TOXINS: INTERACTIONS WITH MAMMALIAN DETOXIFICATION ENZYMES.

H. Hong, P. K. Lam, W. Wang and D. P. Haigh. *Biology Hong Kong University of Science and Technology, Kowloon, Hong Kong and 2Biological Chemistry, City University of Hong Kong, Kowloon, Hong Kong.

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

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

M. E. Finkelstein, K. A. Grauman, D. A. Croll, B. R. Teshy and D. B. Smith. *Environmental Toxicology, University of California, Santa Cruz, CA and 1Department of Biological Sciences, Wright State University, Dayton, OH.

There is a need to develop sensitive biological markers of contaminant-induced effects in wildlife. Immune function may prove a useful marker for toxic effects because its complexity renders it sensitive to contaminant exposure. However, there are limitations in collection and transport of immune cells in the field. Here we report the development of methods to assess immune function in wild birds, using cryo-preserved chicken peripheral white blood cells (WBCs). We determined that cryo-preservation caused a slight but measurable decrease in cell viability (99% +/- .25% for fresh cells and 98% +/- 25% for cryo-preserved cells (n=8)). There was no reduction of viability between cells that were cryo-preserved for less than 10 days (88% +/- 4.5E) and greater than 50 days (95% +/- 15E). Importantly, cryo-preserved cells responded well to tests of immune cell function (mitogen-induced cell proliferation and macrophage phagocytosis). To validate the use of cryo-preserved white blood cells to assess contaminant effects, we used these tests on cells exposed in vivo to the poly aromatic hydrocarbon 9, 10, Dimethyl-1, 2-Benzanthracene (DMBA), a well-known immune suppressor. We found that DMBA significantly (p<0.05) reduced the ability of cells to proliferate and ingest yeast. We tested the applicability of these methods on wild American Coots (Fulica americana) by cryo-preservation WBC for a period of up to 10 months (average viability of 75% +/- 13SD, n=10) and performed immune function tests (proliferation response ranged from 22% to 583% above the non-stimulated cells and the phagocytic ability of macrophages ranged between 15-88%). This research is valuable because it provides a technique to collect samples non-lethally with a single blood sample, as well helping to establish the use of immune function for the assessment of contaminant exposure.

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

J. J. Deska, L., Y. Chung, B. B. Jarvis and H. Tak. *Food Science and Human Nutrition, Michigan State University, East Lansing, MI, 2Institute for Environmental Toxicology, Michigan State University, East Lansing, MI, 3Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI and 4Department of Chemistry and Biochemistry, University of Maryland, College Park, MD.

Although macrocyclic trichothecene mycotoxins produced by Sphaerobys chartarum have been associated with numerous outbreaks of indoor illness, no simple method exists for the detection of these toxins in environmental samples. The goal of this study was to produce specific antibodies to the macrocyclic trichothecene mycotoxin, satratoxin G (SG), and employ these in an enzyme-linked immunosorbent assay (ELISA). SG was derivatized to its hemiacetal and coupled to bovine serum albumin (BSA-SG) for use as an immunogen. Three rabbits were immunized by intradermal injections of the immunogen followed by a single booster injection at week 18. SG-specific antibodies were detected as early as 4 weeks of vaccination and high titer is detectable 38 wks later. A direct competitive ELISA employing microwell adsorbed SG antibodies and SG-hemiacetal coupled to horse-radish peroxidase (HRP-SG) was developed and it did detect as little as 0.1 ng/mL of SG. The concentrations of SG, satratoxin H, inosatatoxin B, roditin A, and verrucarin A causing 50% inhibition of HRP-SG conjugate binding were 1.3, 5.4, 3.2, 25.1, and 5.25 ng/mL, respectively, indicating that the antibodies were more specific for SG. Assay sensitivity was largely unaffected by methanol content up to 20% in toxin dilutions suggesting that the assay could be applied to methanol extracts of environmental samples. The ELISA was applicable detection of satratoxins.

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

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


1Life Sciences, Midwest Research Institute, Kansas City, MO 64114; 2NIEHS, Research Triangle Park, NC; 3Spencer, M. Cunningham.

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

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

S. J. Park and H. G. Jeong. Pharmacy and Research Center for Proteinaceous Materials, Chungnam University, Kwasung, South Korea.

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

325 EFFECTS OF THE HORMONAL GROWTH SUPPLEMENT TRENBOLEONE ON REPRODUCTIVE ENDOCRINOLOGY OF THE FEMALE MINNOW.

G. T. Ankley1, K. M. Jensen1, M. D. Kahl1, E. A. Makynen1, M. W. Hornung2, J. J. Korte1 and L. E. Gray1. 1Mid-Continental Ecology Division, USEPA, Duluth, MN and 2Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.

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

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


Atlantic Ecology Division, USEPA, NHEERI, Narragansett, RI.

Estriol (E1), ethynylestradiol (EE2) and estrone (E1) are steroid estrogens that are released into the aquatic environment in sewage treatment effluent. To determine whether these estrogens impact reproductive parameters in a model fish species, actively-reproducing cunner (Tautogolabrus adspersus) were exposed in the laboratory by implanting estrogen subcutaneously in a slow-release matrix. Four tanks with 3 females and 2 males each were used per treatment. Treatments were control (matrix only) and 0.05, 0.5 or 2.5 mg/kg of each estrogen. Egg production (per gram female), viability and fertility were determined daily. Pre-exposure egg production was not significantly different among experiments, averaging 285 eggs/day. Pre-exposure egg viability ranged from 17% to 28%, while egg fertility ranged from 0% to 100%. Cunner exposed to E1, egg production in the high treatment was 108±22 compared with 174±46 in control, but this difference was not significant. In fish exposed to EE2, egg production in the high treatment (28±22) was significantly lower than in control (107±28). E2 treatment gave no significant differences in egg production, although egg production in the high (99±82) and medium (104±54) significantly was lower than low (188±65) or control (184±104) treatments. Neither mean viability nor fertility was significantly different than control in any estrogen treatment. Although (CSI) was lower in females treated with the 2.5 mg/kg concentration of all estrogens than in other treatments, the decrease was not significant. Average concentrations of vitellogenin (vg) in control males was not significant. The three experiments ranged from 0.0 to 0.5 mg/ml plasma. In males from the highest concentration of EE2, average concentration of plasma vg (480 mg/ml) was approximately four times that in the high E2, treatment (12 mg/ml) and nine times that in the high E1 treatment (53 mg/ml). Overall, only EE2 significantly increased daily egg production of females in control. All estrogens elevated plasma vg in males, but EE2 notably more so than the others.

327 ALTERATION OF F-GLYCOPROTEIN (FGP) EXPRESSION IN CATFISH BY SELECT ENVIRONMENTAL TOXICANTS.

K. M. Kleine, P. Uppa, E. Holmes and Y. Zhang. Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

Sponsor: M. James.

Pgp has been shown to modulate xenobiotic biotransformability and facilitate excretion by efflux transport. Although Pgp in aquatic species is known to be up and down regulated in the environment the etiology of these changes have not been clearly defined. This study examined Pgp protein expression in catfish following exposure to chemical agents eliciting endocrine disruption, and oxidative stress as well as representative polysaccharides and flavonoids. Separate groups of catfish were exposed to doses of Pgp for 6 days with 2mg/kg of nonylphenol ethoxylate (NPE), quercitin (Q), hydrogen peroxide (H2O2), methoxychlor (MC) or control vehicle(C). Plasma membranes of liver and intestinal sections were prepared by selective differential centrifugation methods and subjected to electrophoresis, western blotting and immunostaining with the Pgp, C219 monoclonal. Pgp was visualized and then quantitated by densitometry. Hepatic Pgp concentrations were significantly higher than controls for NPE and were significantly lower for the HP treated fish. These changes represented 46% higher and 39% lower values than controls, respectively. Treatment with Q or MC did not result in significant changes in Pgp for the liver. Enriched brush border membranes from hepatic and distal intestines demonstrated a significantly lower Pgp levels (13.2-211 fold) than livers for all treatments including controls. No significant differences in proximal and distal intestines Pgp concentrations were noted for all treatments. These results demonstrate a differential expression of Pgp between the liver and intestine as well as suggest that select xenobiotics or conditions they elicit may alter expression of catfish Pgp, particularly in the liver. (Supported by NIEHS ES07375).

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A POTENTIAL WHITE STURGEON HEPATIC CYTOCHROME P450 3A WAS CORRELATED WITH LIVER P450 DDE AND PLASMA TESTOSTERONE IN WILD WHITE STURGEON.


White sturgeon (Acipenser transmontanus) were experiencing poor reproductive success in some portion of the Columbia River Basin (USA) which may be related to organochlorine contaminants. Previous studies by our group showed that liver, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p'-DDE) concentrations were inversely related to plasma androgens in reproductively immature male white sturgeon. This study examined the correlation between the putative white sturgeon hepatic cytochrome P450 3A, liver p,p'-DDE, and plasma androgens in reproductively immature male white sturgeon collected from the Columbia River, USA. Liver and plasma were collected from reproductively immature male white sturgeon commercially caught in the Columbia River. Individual levels of chlorinated pesticides and PCBs were measured in liver by GC-ECD, plasma androgens were measured by RIA, and the putative white sturgeon hepatic cytochrome P450 3A was measured in microsomes by western blotting using a polyclonal antibody generated against rainbow trout LCM3 (32A7). The organochlorine measured, p,p'-DDE, was detected most frequently and at the highest concentrations. The putative cytochrome P450 3A was positively correlated with p,p'-DDE. Plasma androgens were inversely correlated with p,p'-DDE and the putative cytochrome P450 3A. These data indicate the existence of a cytochrome P450 3A in white sturgeon and that, p,p'-DDE may induce the putative white sturgeon hepatic cytochrome P450 3A and reduce plasma androgen concentration via increased metabolism. However, additional studies are needed.

ENVIROMENTAL INDUCTION OF CYPIA- AND CYP2KI-LIKE PROTEINS IN TWO TROPICAL FISH SPECIES BY PRODUCED FORMATION WATER ON THE NORTHWEST SHELF OF AUSTRALIA.

M. L. Hassel, M. F. Johnston, and S. Codii.

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

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

A. M. Doi, R. T. Pham and E. P. Gallagher.

Environmental Toxicology Research Program, University of Florida, Gainesville, FL.

A full-length glutathione S-transferase (GST) cDNA was obtained from largemouth bass (Micropterus salmoides) liver total RNA using 5' and 3' systems for rapid amplification of cDNA ends (5' RACE and 3' RACE). The full-length GST cDNA was 977 base pairs (bp) in length and contained an open reading frame of 666 bp, encoding a polypeptide of 222 amino acids. The larval GST exhibited high homology to a theta-like GST (GST-A) previously isolated from plaice (Pleuronectes plaiceus) with high catalytic activity towards 4-hydroxynonenal (4HNE), a toxic and mutagenic breakdown product generated during the peroxidation of polyunsaturated fatty acids. Northern blotting analysis of largemouth bass liver RNA revealed strong expression of a GST-like protein band of approximately 1 kb. In addition, affinity-purified bass hepatic cytosolic fractions displayed high catalytic activity towards 4HNE. In summary, the presence of a highly expressed GST isozyme in bass with extensive sequence homology to plaice GST-A is suggestive of a role of GST in protecting against oxidative damage in this freshwater species. Supported by NIH P42 ES05735.

RELATIONSHIP BETWEEN LIVER OIL: INFRARED SPECTRA AND FISH HEALTH.


Environmental and Computational Chemistry Group, University of Cartagena, Cartagena, Colombia.

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


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

335 MAXIMALLY PERSISTENT PCB CONGENERS IN FISHES AND HUMANS.


We analyzed PCB tissue burdens in humans with intermittent links to PCB contaminated fish. Pattern recognition by principal components analysis was applied to data on Ojibwa native Americans. Great Lake fisheries and non-native Lake Michigan fisheries as well as PCB contaminated fish fillets were analyzed. The products identified by electron-capture-gas chromatography were: chlorinated hydrophobic PCBs consumption data were obtained. No evidence of PCB concentration in fish fillets were different from a healthy population. Local-area micro-ecoregion PCB patterns were the principal determinant of PCB human-contaminant-scan patterns. Among PCB congeners, ortho position is the most frequent position regardless whether they are the game fishermen or the Ojibwa Indians. The pentachlorinated isomers are the most prevalent and the combination of these characteristics, the ortho-chlorination, the pentas and hexachlorinated isomers determine not only the ubiquity/bioavailability, but also the bioaccumulation/biomagnification in all organisms including fish consuming humans. Disclaimer: The conclusions and opinions in this abstract are those of the authors and do not represent affiliated institutions.

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

J. D. Duffield', C. Lambricht', V. Wilson', L. B. Gray', J. G. Guillet' and G. T. Axley'. 1Mid-Continent Ecology Division, USEPA, Duluth, MN; 2Reproductive Toxicology Division, USEPA, Research Triangle Park, NC; and 3University of Florida, Gainesville, FL.

Pulp mill effluent samples from the Fenhollow river in Florida were collected and tested using a bioassay-based fractionation approach to identify the causative agents responsible for androgenic effects in female fish from the stream. An androgenic activity of the effluent and subsequent fractions was 'traced' through the process using CV-1 cells transfected with a human androgen receptor -MLTV-mucin promoter-reporter system (Parka et al. 2001) Toxicol. Sciences. 62:257. The goal of the approach was to separate biologically-active components from non-active components of the complex effluent matrix to facilitate the identification of the chemical(s) responsible for androgenic activity. Initially organic chemicals were isolated from the effluent using a C18 solid phase extraction (SPE) cartridge, which was subsequently eluted with graded concentrations of methanol in water. SPE fractions which exhibited androgenic activity were then fractionated further using high pressure liquid chromatography (HPLC) into twenty-five discrete fractions. In these studies, multiple HPLC fractionation steps were required to adequately simplify samples for chemical identification. The HPLC fractions displaying activity in the CV-1 cell assay were then analyzed using gas and liquid chromatography linked with electron impact, or chemical ionization (both positive and negative ion) mass spectrometry. Androstenedione, a steroid previously identified by other researchers as responsible for androgenicity in this effluent, was found in the effluent sample but not in the active HPLC fractions. Additional mass spectrometry results to date indicate the presence of many steroidal compounds, as well as a few halogenated chemicals in the active fractions. This abstract does not necessarily reflect EPA policy.

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

Q. H. Fely and D. Boy. Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.

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

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


Fumonisin B, (FB), is a water soluble, carcinogenic mycotoxin produced by the fungus Fusarium moniliforme, which is parasitic to corn plants. It is estimated that 90% of the FB, consumed by
livestock is excreted unmetabolized. The objectives of this research are to determine 1) the kinetics of FB, interaction with soil constituents, and 2) If FB, is chemically modified in the soil. Leachate columns were used to determine the movement of FB, through soil matrices comprised of washed sand and 0%, 50%, 75%, or 100% Celery sandy loam soil. The movement of FB, was compared to that of bromophenol blue (BB), a dye that moved freely through the soil columns. FB, contaminated corn screenings or extract containing FB, were placed on the surface of the soil columns. When corn screenings were used as the source of FB, the radicator was attached to the top of the column and water was allowed to saturate the corn screenings (extracting the FB,) and percolate through the column eluting the FB,. The 100% sand columns slightly retarded the efflux of FB, relative to BB but did not appear to chemically alter the FB,. The recovery of FB, decreased with increasing concentrations of Celery sandy loam soil at 0%, 50%, 75% and 100% Celery sandy loam, approximately 80%, 60%, 50% and 20% of the FB, was recovered in the column leachate, respectively. The FB, retained on the 100% Celery sandy loam column was tightly bound as evidenced by the fact it could not be extracted using acetonitrile-water (1:1). However, approximately 40% of the retained FB, was extracted using 5% formic acid/acetonitrile (1:1) indicating that the nature of the interaction was probably ionic. The result of this study indicates that FB, is probably stable in the soil environment and suggests that while sites are tightly bound, under certain environmental conditions FB, could be released, become biologically available and possibly contaminate the water supply.

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

T. C. Mathieson1, S. Li1, C. T. Swann1 and R. L. Erickson1. 1Pharmacology and Division of Environmental Health Res., Univ. of Mississippi, University, MS. 2Mississippi Mineral Resources Inst., Univ. of Mississippi, University, MS and 3Mississippi State Board of Registered Professional Engineers, Jackson, MS.

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

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


Natural processes of Mn accumulation and the legacy of Pb additives in fuel for predicting Mn in the environment are the topics of this study. Data is from alluvial and sediments of the lower Mississippi River Delta and a soil metal survey of metropolitan New Orleans. The "railroad manganese," "industrial manganese" and "dynamic aquifer-stream transfer of Mn" hypotheses were tested. The results support the proposal that interaction between Mn-rich steel whets and rails contribute Mn to the environment. Sediment loads of Mn upstream and downstream from LA industrial corridor were similar (t-value = 0.77) and this data does not support the industrial manganese hypothesis. Data medians of the alluvium surface (59 mg/kg), the aquifer (159 mg/kg) and the riverbank (513 mg/kg) where Mn-rich clay precipitates during aquifer discharge support the aquifer-stream transfer hypothesis. Soils along the urban road, respectively, medians of 216 and 85 mg/kg for Mn, and 172 and 85 mg/kg for Pb. The survey of metropolitan New Orleans indicates an increase from rural to interior urban communities by a factor of 4 for Mn and nearly 3 orders of magnitude for Pb. Assuming 1999 US on-road fuel use at 3.3 mg of Mn per L, 5 thousand metric tons of Mn would be consumed. If 1% of the Mn added to fuel remained, 550 metric tons of Mn would become aerosols annually, and 87%, or 4, 350 metric tons would remain in the engine. The 1999 TRI for Mn shows 10 metric tons as stack releases and 370 metric tons as total emissions. In 1999, 60% of vehicle travel occurred in urban and 39% occurred in non-urban areas. Annually, Mn in fuel would emit 390 and 260 metric tons respectively in urban and non-urban areas. Mn would mimic the pattern for Pb. Release of Mn aerosol cleanup would be dominated by pollution with Pb regulations. On Executive Order 12898 regarding Federal redress of environmental justice in minority and low-income populations. Funded by ATSDR/HHF cooper- 
agree-ment #U50/AT0398948 to Xavier University of LA.

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


Contamination of building structural elements which may be a result of fuel oil spills, catastrophic flooding etc., results in the release of chemicals into the air over the course of years. An efficient method of reducing the human exposure to the hydrocarbons is suggested based on bioremediation, i.e., application of live microorgan- isms on polluted building materials, such as wood and concrete. The purpose of this bench-scale study was to evaluate the feasibility of bioremediation for the removal of hydrocarbons using naphthalene and n-hexadecane as model pollutants. Radiolabeled hydrocarbon was applied on standard pieces of building materials followed by the application of different bioremediation treatments. The degradation efficiency was monitored by scintillation counting. Two kinds of experiment were conducted: shaking the samples with biomass in mineral medium (shaking flask procedure) and attaching the building material samples on the agar medium where the biomass has been grown (overlay procedure). The observed similarity of pollutant removal kinetics for shaking flask and overlay procedures indicated that the rate-limiting step appears to be diffusion of the hydrocarbons within the pores of a building ma- terial. Moisture content of the samples had a critical influence on the degradation efficiency. Water was shown to hinder the diffusion of hydrocarbons in wood and concrete. This effect was used to accelerate the pollutant removal by adjusting the protocols for biomass application. Among the factors influencing hexadecane re- moval efficiency, the following conditions of biomass growth (prior to their application) were found to be critical: the time and temperature of cultivation, composition of nutrient medium, and the regime of re-inoculations. Preventing the biomass starvation resulted in a significant increase of pollutant removal. For 6-mm thick wood and one gram concrete samples, maximum hexadecane removal achieved without applying new biomass was 85-93%, which required ca. 2 weeks.

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MINERALOGICAL AND GEOCHEMICAL VARIATIONS AMONG ASBESTOS STANDARDS.


It is well known that exposure to asbestos mineral dust is associated with lung diseases such as asbestosis, malignant mesothelioma, and bronchogenic carcinoma. Past attention primarily focused on human exposure and health effects of commercial asbestos. In recent years, there has been renewed recognition of the potential deleterious health effects of asbestos that occur as accessory minerals in rocks (i.e., serpentinite, a common rock in US and) and in some mineral commodities used commercially (i.e.,vermiculite, talc). Within these contexts, many in vitro studies have shown asbestos to be cytotoxic and mutagenic, yet the mechan- isms responsible for this are not fully understood. These questions can be best answered through continued and increased collaborative research between the earth science and health-related communities. One example of this is the mineralogical and geochemical characterization of individual asbestos family members. The USGS has assembled sets of asbestos standards, which have been previously used in toxicologi- cal studies (Standard sets:5 amosite, 4 anthophyllites, 6 chrysotiles, 5 crocidolites, 4 tremolites). Presently, we have identified the mineralogical and geochemical charac- teristics for each set of asbestos standards. Chemical analyses indicate that ele- mental (i.e.,Ca, Co, Cr, Fe, Ga, Li, Mg, Mn, Ni) content varies between the different asbestos minerals. Variations within the standards of the same asbestos mineral sets are present. X-ray diffraction and SEM also indicate differences in asbest- os habit (crystal shape, cleavage pattern) and the presence of other minerals as contaminates between different asbestos standards and within the sets of asbestos standards. These variations between the asbestos standards or those within the sets.
DILUTED DIESEL EXHAUST EMISSIONS DO NOT INDUCE CHANGES IN PERIPHERAL BLOOD MICRONUCLEI LEVELS IN SUBCHRONICALLY EXPOSED MALE AND FEMALE A/J MICE.

M. D. Reed. Lovelace Respiratory Research Institute, Albuquerque, NM.

Focus has been directed toward the cancer risk associated with diesel emissions. Contribution to air pollution in both occupational and environmental settings. As one component of the National Environmental Respiratory Center's long-term goal to assess the carcinogenic potential of many anthropogenic source emissions, we determined the clastogenic ability of subchronic exposures of diesel emissions to induce peripheral blood micronuclei in the strain A/J mouse. One-hundred male and one-hundred female A/J mice (10 male and 10 female mice per group) were exposed to diesel emissions generated from one of two 2000 Cammins B series engines operated on the heavy duty test cycle with EPA certification diesel fuel. Chamber concentrations were determined by diesel exhaust particulate matter and maintained at 0, 30, 100, 300, and 1000 μg/m³ respectively. Exposures were conducted for 6½ days, 7 days a week, for a period of 6 months. Micronuclei were assessed in hematoxylin and eosin-stained peripheral blood from each animal by flow cytometry (~20,000 reticulocytes counted per animal). Levels of micronuclei in clean air-exposed, 6-month-old mice were consistent with previously published reports in juvenile and age-matched A/J mice (within 3 standard deviation of the mean). As analyzed by one-way ANOVA and the Student's t-test for post hoc comparison to control, there were no differences between the mean levels of micronuclei in control and exposed mice (p=0.05). These findings indicate that exposure of A/J mice to diesel emissions diluted up to 1000 μg/m³ does not induce clastogenic changes in micronuclei levels as measured in peripheral blood. Research conducted by the National Environmental Respiratory Center (NERC) with support from multiple government and industry sponsors, including the USEPA. This abstract is not intended to represent the views or policies of any NERC sponsor.

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


Studies with respirable quartz using naked DNA as a target suggest that hydroxyl radical formation by the particle itself may play a role in the DNA-damaging properties of quartz. However, it is not known whether this pathway is important for DNA damage in alveolar epithelial cells which are considered as target cells for quartz carcinogenesis. Therefore, we determined DNA damage by quartz or hydrogen peroxide in A549 human and RLE rat alveolar epithelial cells using the alkaline comet assay. The hydroxyl radical-generating capacity of the quartz was analysed by electron spin resonance (ESR), and by immunochemical analysis of the hydroxyl radical-specific DNA lesion 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the epithelial cells. Quartz particles as well as hydrogen peroxide were found to cause a dose-dependent increase in DNA strand breaks in both cell lines. DNA damage by quartz was significantly reduced in the presence of the hydroxyl radical scavenger mannitol or DMSO. The involvement of hydroxyl radical was also demonstrated by ESR measurements, and by the ability of the quartz to induce formation of 8-OHdG in both cell types. In conclusion, our data show that quartz elicits DNA damage in human and rat alveolar epithelial cells, and indicate that these effects are driven by intrinsic hydroxyl radical-generating properties of the particles.

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

Q. Meng,1 R. E. Henderson,1 M. Bauer and V. F. Walker.1 Lovelace Respiratory Research Institute, Albuquerque, NM and 'School of Public Health, State University of New York at Albany, Albany, NY.

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


Benzene is a genotoxic carcinogen that induces chromosomal breaks as a primary mode of genotoxicity in the bone marrow (BM). We showed that chronic exposure to inhaled benzene induced a p53-regulated DNA damage response in mouse BM. We investigated the nonhomologous end joining (NHEJ) pathway of DNA double strand break (DSB) repair in benzene genotoxicity. Quantitative RT-PCR was used to determine Ku80 mRNA levels in the BM of C57BL/6 mice exposed to inhaled benzene (100 ppm) for 15 weeks, from which benzene would induce expression of genes, such as Ku80, involved in NHEJ. However, Ku80 mRNA levels in benzene-exposed mice were not significantly altered. Next, we compared the genotoxic effects of inhaled benzene on C57BL/6 and 129/SvJ mice which differ at the Pkde locus encoding the catalytic subunit of DNA-PK (DNA-PKcs) that binds to the Ku70/Ku80 heterodimer during NHEJ. C57BL/6 mice have a wild-type allele at Pkde while 129/SvJ mice have a Pkde functional polymorphism that leads to diminished DNA-PK activity and enhanced apoptosis in response to radiation-induced damage. We expected mice with deficient DNA-PK activity to be more susceptible to genotoxic effects of inhaled benzene. Mice were exposed to 0, 10, or 100 ppm benzene for 6, 12, or 24 days for 2 weeks. Both strains had decreased WBC and lymphocyte counts in the blood with increasing doses of benzene. Flow cytometric cell cycle analysis on BM cells stained with propidium iodide revealed no G1 arrest in either strain at any dose of benzene. Genotoxicity was assessed by a flow cytometry assay that distinguishes micronucleated mature normochromatic erythrocytes (MN-NCE) from micronucleated reticulocytes (MN-RET) in BM. We observed comparable increases in MN-NCE and MN-RET populations in both C57BL/6 and 129/SvJ mice following benzene exposure, suggesting the difference in Pkde alleles does not affect benzene-induced genotoxicity. The data indicate that NHEJ, mediated by the Ku heterodimer and DNA-PKcs, is a significant pathway in the repair of benzene-induced DSB.


Chronic benzene is hematotoxic, genotoxic, and carcinogenic in humans and rodents, causing a variety of hematopoietic disorders. The enzyme systems involved in activation and detoxification reactions of benzene metabolism are likely genetic determinants of individual variability and risk in response to benzene. NQO1 catalyzes the detoxication of 1, 4-benzene, a common ultimate toxic benzene metabolite. mEH metabolizes benzene oxide to dihydrodiol, which can then be converted to catechol or trans-1-muconaldehyde, both of which can lead to genotoxic metabolites. Our hypothesis is that the NQO1 and mEH pathways are critical to benzene-induced toxicity. We exposed NQO1−/− and mEH−/− mice to inhaled benzene (10 or 100 ppm) for 2 weeks and determined the hematotoxicity and genotoxicity relative to the 129/SvJ (wild-type WT) mice. Micronucleated (MN) polychromatolytic erythrocytes (PCE) and immature RBC levels in the blood were used as a measure of genotoxicity. In preliminary studies, WT mice were almost twice as sensitive to benzene-induced genotoxicity (2.7% MN-PCE, p<0.01) compared to NQO1−/− mice (1.3% MN-PCE, p=0.01). mEH−/− mice were not sensitive to benzene (0.9% MN-PCE) compared to WT mice (3.5% MN-PCE, p=0.01). P21 mRNA gene expression measured by quantitative RT-PCR in bone marrow was used as a genotoxic biomarker of the p53 DNA damage response pathway. WT and NQO1−/− mice had a 3.7-fold increase at the 100 ppm dose over unexposed controls in p21 expression in response to benzene compared to a 1.4-fold increase in mEH−/− mice. p21 induction in WT and NQO1−/− mice suggests the p53 pathway is activated. These preliminary results suggest that NQO1 deficiency does not lead to increased benzene-induced genotoxicity and that mEH deficiency results in protection against benzene-induced genotoxicity.


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

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


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

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

I. D. Claxton, T. J. Hughes and K. T. Chung. "Environmental Carcinogenesis Division, USEPA, Research Triangle Park, NC and "Biology Department, University of Memphis, Memphis, TN.

Benzidine (Bz), 4-amino-phenyl (ABP), 3', 3'-dichlorobenzidine (DCBz), 3', 3'-dimethylbenzidine (DMBz), 3', 3'-dimethoxybenzidine (DMOBz) and the benzidine-congener dye trypan blue (TB) produce primarily framshift mutations in Salmonella typhimurium. However, the base-substitution strain TA100 also responds to these compounds when 59 is present. DNA sequence analysis by other investigators shows that ABP induces frame-shift, base-pair, and complex mutations. Also, an uninduced hamster liver S9 preparation with glucose-6-phosphate dehydrogenase (G-6-PDH), FMN, NADH and 4 times glucose 6-phosphate gives a stronger mutagenic response than the conventional plate incorporation with a rat S9 activation mixture for all the compounds tested. Using the base-specific tester strains of S. typhimurium (TA7001 - TA7006) with the above reductive mutagenic activation system, we surveyed these compounds for their ability to produce specific base-pair substitutions after reductive metabolism. Bz was weakly mutagenic in TA7005 (0.04 revertants/μg); ABP was mutagenic in TA7002 (1.4 revertants/μg), TA7004 (0.6 revertants/μg), TA7005 (2.98 revertants/μg) and TA7006 (0.4 revertants/μg). DCBz was weakly mutagenic in TA7004 (0.01 revertants/μg). It was concluded that benzidine induced some GC→AT and TG→TC transitions. Because DCBz and DMOBz and TB were not mutagenic in this base-substitution and then detection system, their mutagenic activity was attributed strictly to frameshift mechanisms. This is an abstract of a proposed presentation and does not necessarily represent the views of the USEPA.
INDUCTION OF DNA DAMAGE AND REPAIR BY THE MYCOTOXIN OCHRATOXIN A IN DIFFERENT CELL TYPES.


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

GENOTOXIC EFFECTS OF SOY ISOFлавONES AND TWO MAJOR OXIDATIVE METABOLITES.

S. E. Kulling, C. E. Ruefer, A. Hartwig and M. Metzler. Institute of Nutritional Physiology, Federal Research Center for Nutrition, Karlsruhe, Germany and Institute of Food Chemistry and Toxicology, University of Karlsruhe, Karlsruhe, Germany.

The isoflavonoid phytoestrogens daidzein (DAI), genistein (GEN) and glycitein (GLY) are major dietary components of soybeans. There is a growing scientific interest in phytoestrogens due to their potential beneficial properties for human health. Isoflavonoid intake seems to be inversely associated with the incidence of hormone-dependent diseases, e.g. breast cancer. Recently, the oxidative metabolism of DAI and GEN was investigated in our Lab. Both isoflavones are converted by cytochrome P450 enzymes to several catabolic metabolites, e.g. 6, 7, 4'- and 7, 3', 4'-diarylhydroxysoyflavone (6, 7, 4'-OH; 7, 3', 4'-OH), which may represent a metabolic activation process. Using the comet assay, we have investigated the potential of these compounds which may cause DNA damage. The extent of DNA strand breaks (SB) and oxidative DNA modifications (recognized by the purified bacterial repair enzyme formamidopyrimidine-DNA glycosylase, Fpg) was assessed in HeLa-S3 cells. As expected from results of the literature DAI does not cause any DNA damage in contrast to GEN, which leads to high amounts of SB. GLY induces a small number of SB, 6, 7, 4'-IF and 7, 3', 4'-IF cause small amounts of SB with a maximum after 2 h incubation time (as GEN and GLY) and high amounts of Fpg-sensitive sites at the end of incubation (24 h). The DNA-damaging potential of GEN is probably associated - as described in the literature - with the inhibition of topoisomerase II. 6, 7, 4'-IF and 7, 3', 4'-IF cause oxidative DNA damage and SB probably by generating reactive oxygen species via "redox cycling" and forming DNA adducts, respectively. Supported by Deutsche Forschungsgemeinschaft (Grant Ku 1679/5-1).

GENETIC TOXICOLOGY PROFILE OF INACTINE PEN110.


The V. L. Technologies process for the purification of blood involves removal of lymphocytes followed by inactivation of viruses, bacteria, protozoa and residual lymphocytes with the compound PEN110. PEN110 achieves pathogen inactivation by modification of nucleic acid bases to disrupt replication of the genome by polymerase enzymes. During incubation of red blood cells, PEN110 has the ability to inactive free, cell associated, and integrated HIV. Red blood cells are then extensively washed to remove PEN110 and plasma proteins. The genetic toxicology profile studies presented here are a key segment of the work to assure that exposures to any residual PEN110 will be without adverse effects. PEN110 is positive without metabolic activation in the rat hepatocyte in vitro unscheduled DNA synthesis (UDS) assay, in Ames base pair substitution strains TA1535 and TA100, and in the mouse lymphoma assay. The spectrum of genotoxic activity of PEN110 is, however, narrow, apparently producing only base pair mutations and showing no activity in chromosome assays or the whole animal. PEN110 was negative in the in vivo in vitro UDS assay, in the Ames frame shift strains TA98, TA100 and WP2uvrA, in the chromosomal aberration assay in CHO cells, and in inducing chromosomal aberrations in freshly drawn human blood peripheral lymphocytes. No response was seen in the in vivo in vitro UDS assay by i.v. administration at a dose giving an initial nominal blood concentration of over 200 times that which had been demonstrated to respond in culture. This indicates the presence of a rapid, direct genotoxic pathway that clears PEN110 before it can mutate human DNA. PEN110 was negative in the in vivo in vitro UDS assay at a dose approximately 12, 000 fold over the anticipated human dose following a 1 unit transfusion. These quantitative genetic toxicity studies indicate that the trace amount of residual PEN110 in purified blood products represents an insignificant risk to the patient.

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

W. J. Mackay and J. Brumfield. Biology and Health Services, Edinboro University, Edinboro, Ph. Sponsor: W. Mackay.

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

THE POTENTIAL GENOTOXICITY OF BRUVINDIN.

J. Brigavoidi. Research Toxicology Centre, Pomezia, Italy.

Bruvindin (E)-5-(2-Bromovinyl)-2-deoxyuridine, BVUDU) is a nucleoside analogue with selective activity against varicella zoster virus (VZV) and herpes simplex virus type 1 (HSV-1). It has recently received marketing approval in Germany for the early treatment of VZV infections. Bruvindin is selectively phosphorylated to the mono- and diphosphate by viral enzymes. This leads to accumulation of Bruvindin within virus infected cells. The monophosphate is further converted to the triphosphate by cellular enzymes. The triphosphate interacts with viral DNA-polymerase acting either as a substrate for or an inhibitor of the enzyme. Although no bruvindin incorporation into non-virus infected cells has been detected, the mechanism of anti-viral action does suggest scope for some mutagenic or clastogenic potential. A comprehensive battery of in vitro and in vivo tests to further modify the toxicity of the nucleoside has been performed. Data presented from investigations in vitro assayed: a Salmonella typhimurium reverse mutation (Ames) test; an HGPRT test in CHO cells; two chromosome aberration studies in human lymphocytes and an unscheduled DNA synthesis (UDS) study. Bruvindin was consistently negative for gene mutation and did not induce UDS. Clear increases in the frequency of chromosome aberrations were seen in human lymphocytes after 44 hours of continuous treatment in the absence of metabolic activation only. Data are also presented from the following in vitro assay: SOT 2002 ANNUAL MEETING 73
in vitro assays: a UDS assay in the rat (this also included assessment of replicative DNA synthesis); two micronucleus studies in mice and a spot test in mice. These assays were all negative for genotoxicity, although a treated rat related increase was seen in replicative DNA synthesis. The data indicate that brivudin has no potential for mutagenic activity. The increase in chromosome aberrations is concluded to be of no biological significance for the human safety assessment of brivudin. In addition, the data support the argument that a non-genotoxic mechanism is responsible for the reported induction of liver tumours in rats, following oral administration of brivudin for 1 year (Sembler, et al., 1987, J. Am. Coll. Toxicol. 6, 557).

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

P.J. Duerksen-Hughes and M. Filippova. Biochemistry and Microbiology, Loma Linda University, Loma Linda, CA.

Our laboratory has previously developed a mammalian, in vitro assay for genotoxicity based on the ability of cells to increase their level of p53 in response to DNA damage. Cultured cells are treated with the test substance, and at defined times following treatment, are harvested and lysed. The lysates are then analyzed for p53 by ELISA. An increase in cellular p53 following treatment is interpreted as evidence for DNA damage, and a lack of such an increase is interpreted as evidence of no significant DNA damage. To determine whether this system would work in cultured human cell lines, as well as in mouse cells, we tested several human cell lines known to express wild-type p53 for their ability to increase their p53 levels following DNA damage. Five such cell lines, U27, W138, ZR-75-1, MCF-7 and U2OS were identified. To increase the speed and economy of this assay and to improve its ability to function in a high throughput application, we then developed indicator cells that can identify DNA damage without the need for cell lysis. Plasmids coding for the p53-responsive element fused to the gene coding for the reporter protein secreted alkaline phosphatase (SEAP) were stably transfected into human U2OS cells. Several individual clones were then isolated and characterized. When treated with the genotoxin mitomycin C, these cells increased both their intracellular level of p53 and the level of SEAP by several-fold, confirming their usefulness as indicator cells.

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


Various environmental factors can be a cause of DNA damage, and accordingly it is conceivable the damage is accumulated with age particularly in the organs exerting high function in the body, i.e. the liver and kidney. This study examined the correlation between aging and DNA damage in the rat kidney. In addition, the vulnerability of DNA to a chemical carcinogen in the kidney was compared between young and aged rats. The detection of DNA damage was conducted with a comet assay (single cell gel electrophoresis). Male F344 rats at 9 weeks and 9 months old were treated with a vehicle (0.5% carboxymethylcellulose sodium: CMSC) or chemical carcinogen (methyl methanesulfonate: MMS at 80 mg/kg). Three hours after dosing, the kidney was homogenized to make cell suspension and specimens were prepared. The specimen was electrophoresed under alkaline conditions by applying and unwinding DNA and then stained with ethidium bromide. Fifty nuclei from each specimen were observed under a fluorescence microscope to measure DNA migration as an index of DNA damage. The average values of DNA migration were statistically compared. In the vehicle control groups, DNA migration in the 9-month-old rats was 16.75 ± 1.80 µm which was statistically greater than the DNA migration (7.96 ± 0.74 µm) in the 9-week-old rats. In the MMS-treated groups, DNA migration in the 9-week-old rats was 29.46 ± 3.28 µm, whereas DNA migration in 9-month-old rats could not be determined due to severe DNA damage. The comet image in the MMS-treated groups at 9 months was similar to a typical image of apoptosis. This preliminary study suggests that spontaneous DNA damage and vulnerability of DNA to a chemical carcinogen in the rat kidney increase with age. The higher vulnerability in the 9-month-old rats may be attributable to an age-dependent increase in accumulated oxidative damage of DNA.

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


The Ames Test evaluates the mutagenic potential of test chemicals by analyzing their effect on histidine (his) requiring strains of Salmonella typhimurium. Salmonella strain TA102, which causes a base substitution mutation, has its his gene located on a multicopy plasmid, pAQ1. After strain TA102 was exposed to the oxidative mutagen, ethylene oxide, pAQ1 was isolated (Miniprep kit, Qiagen) and the his gene was extracted from the plasmid by restriction digest (Stratagene). The restriction fragments containing the his gene were analyzed by denaturing HPLC (Transgenic WAVE system), and mutations were detected based on the ability to visualize the formation of heteroduplexes. When a sample contains one uniform sequence of DNA, a single molecule can form as two complementary strands (anneal, homoduplex). However, a sample containing two DNA molecules that differ by as little as a single nucleotide, can result in the formation of two homoduplexes and two heteroduplexes. Denaturing HPLC separates these molecules with the heteroduplexes eluting first. If both samples are identical in sequence, no heteroduplex will form and only one peak will be detected. Analysis of the digest of the pAQ1 plasmid by electrophoresis indicated that after 1h, 37°C, the 322bp fragment could be detected. Samples from each of the mutants were mixed with the wild type at a ratio of 1:1 and incubated at 95°C for 5 minutes. After finding a peak at 25°C for 45 minutes. The fragments were subjected to a temperature titration (50-62°C) and were analyzed on the WAVE system. The wild-type fragments (homoduplexes) as well as the mutant fragments (heteroduplexes) were detected. Both the hetero- and homoduplexes eluted at the predicted time based on the method for mutation detection using the WAVE software. These modified Ames assay can be used to detect mutations using a high throughput, accurate and inexpensive procedure. Evaluation of other Salmonella strains, genomic DNA and PCR products are studies which are currently in progress.

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

J. Kitching, M. G. Wing, G. Clare, K. May and C. K. Atterwill. Experimental Biology, Huntington Life Sciences, Huntington, United Kingdom.

Regulatory genetic toxicology produces 20-25% in vitro positive results per year. We compared the Sos/umu assay with a mutagenicity screen with the corresponding Ames result. The umu assay uses S. typhimurium strain TA1535/60102 in which lac-Z is under the control of the SOS repair genes. Compounds were tested up to 1 mg/ml (approximately equal to the maximum dose in the Ames test, 5 mg/plate). The Ames test was performed using standard regulatory methods with S. typhimurium strains TA1535, TA1537, TA98 and TA100 and Escherichia coli strain CM891. A number of mutagens (Allan: B, D, 5-nitro-2-aminoimidazole, methyl methanesulfonate, 2-aminoantraacarcene, nitrosofuran, BENZO(A)PYRENE, methyl nitro-nitrosoguanidine, daunamycin, 2-aminofluorene, 9-aminocaridine, 2-nitrofluorene and sodium azide) were tested along with non-mutagentic toxic compounds (DMSO, ampicillin, tetracycline, amphotericin B, penstrep and gentamycin), together with 12 proprietary compounds concluded as regulatory Ames mutagens. All were tested in the presence and absence of rat liver S9 mix. The data correlated well with the Ames test, with all 12 of the classic mutagens tested were detected and no false positives found. Of the 12 proprietary compounds the umu assay detected 9. Those that were not detected were weakly positive in the Ames test and of them in CM891, the widely used Salmonella reverse mutation assay correctly did not induce the SOS repair pathway. On the whole the umu assay ranked well with that for the Ames test. However, one anomaly was a proprietary compound that was weakly positive in the Ames test and strongly positive in the umu assay. There did not appear to be any difference in sensitivity of the umu assay between frameshift and base pair substitution mutations. It is concluded that it is possible to screen out the majority of genotoxins as a very early stage of compound development. This will give vital information, which if integrated with data from other lead optimisation screens will improve the process of lead candidate selection.

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

R. H. Schiestl and R. J. Brennan. Pathology and Environmental Health, UCLA, Los Angeles, CA and OSIR Pharmaceuticals, Uniondale, NY.

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


Incorporating genotoxicity endpoint(s) into subchronic toxicology studies can potentially reduce animal usage. Recent data suggests that rat peripheral blood may be a sensitive compartment for highlighting chemical-induced cytogenic damage expressed as micronuclei (MN). Many of these experiments have utilized conventional microscopy-based inspection to score MN events. An experiment was conducted to evaluate whether a method for scoring MN by high-speed cell sorting (HCS) could be substituted for that achieved by microscopy. This HCS method utilized an immunohistochemical reagent (anti-Cd71-FITC), RNase, and propidium iodide to differentially stain four cell populations: normochromatic erythrocytes, normochromatic erythrocites with MN, reticulocytes and reticuloocytes with MN (commercially available as MicroFlow®PLUS)). For this experiment, rats were treated with vehicle (saline) or a range of methyl methanesulfonate (MMS) concentrations 5 days per week for 4 weeks. Blood samples were drawn initially on Day 0 and then on Days 2, 3, 4, 11, 18 and 25. MN frequencies were determined by analyzing 20,000 young reticulocytes per blood sample. Instrumentation and calibration procedures utilizing rat bone marrow and a biological model were employed, and described. Vehicle treatment showed no change in MN-RET frequency over the experimental timeframe, irrespective of scoring methodology. For MMS treated animals, the MN-RET frequency increased in a time and dose-dependent manner. While the average MN-RET values were similar for both scoring procedures, less variation was evident with the HCS-based measurements. Note that the HCS-based data acquisition was achieved in a fraction of the time (1/10) of the microscopy-based measurements.

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


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

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ENZYMATIC ACTIVITIES OF WERNER SYNDROME PROTEIN ON A TELOMER TYPE SUBSTRATE.

S. Theodore and D. K. Orren. Graduate Center For Toxicology, University Of Kentucky, Lexington, KY. Sponsor: M. Vose.

Werner Syndrome (WS) is a hereditary disorder that is characterized by early onset of aging symptoms and increased frequency of age-related diseases including cancer and atherosclerosis. WS is caused by mutations in a gene that codes for the Werner protein (WRN) that contains both DNA unwinding (helicase) and exonuclease activities suggesting a role in DNA metabolism. WRN belongs to the RecQ family of helicases that are involved in maintaining genomic stability. Consequently, defects in WRN lead to increased genomic instability characterized by chromosomal deletions, insertions, deletions and the accelerated loss of telomeres, the telomerase repeating sequences at the ends of chromosomes. In this context, WS serves as a good model for examining the relationship between accumulation of genetic changes induced by endogenous and environmental agents during lifetime and the degenerative processes that occur during normal aging. In most normal somatic processes, cells, telocytes shorten with each round of cell division, essentially leading to cessation of cell division known as cellular senescence. In WS cells, telocytes shorten at a much faster rate, leading to premature cellular senescence and suggesting a possible role for WRN in telomere length maintenance. Recent studies indicate that each chromosome forms a specialized structure known as the T loop in which the 3'single-stranded telomeric end is inserted back into the double-stranded telomeric region. We have constructed model DNA substrates that mimic the T loop structure of telomeres and have carried out experiments to study the activities of WRN on these substrates. DNase I footprinting indicates that WRN binds specifically to the T loop structure. This binding appears to mediate the disruption of the T loop structure. In addition, WRN has a very efficient exonuclease activity on the 3' ends of our T loop substrate. Our findings suggest a potential role for WRN in processing T loop structures during telomere metabolism.
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THE EFFECT OF DIFFERENT DNA DAMAGING AGENTS ON P53 AND MDM2 PROTEINS IN CANCER CELL LINES AND MOUSE SKIN.

L. Laatik, R. Serpi, U. Puistola1, and K. H. Vahakangas,1.1 Department of Pharmacology and Toxicology, University of Oulu, Oulu, Finland. 1Department of Obstetrics and Gynecology, University Hospital of Oulu, Oulu, Finland. 1Department of Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland. Sponsor: K. Suelainen.

The production of p53 gene is a transcription factor for several known cancer cell cycle regulation-linked genes. Wild type p53 protein is increased, probably by stabilization, in response to various cellular stress signals, e.g. chemically induced DNA damage. The increase of p53 is thought to be a consequence of disrupted binding of p53 to Mdm2, which is mainly responsible for the degradation of p53. The aim of this study was to find out whether Mdm2 is the cause of p53 increase after treatment with different DNA damaging agents. We used MCF-7 breast adenocarcinoma and A-549 lung carcinoma cells and an in vivo C57BL/6 mouse skin model to study the effects of benzo(a)pyrene, cisplatin, docetaxel, and azidostyroidine (AZT) on p53 and Mdm2 proteins. The amount of p53 and Mdm2 proteins were studied by Western blotting using antibodies DO7 and CM5 for p53 (human and mouse, respectively) and H-221 for Mdm2 (both human and mouse). p53 was induced by benzo(a)pyrene in both cell lines and in mouse skin. Cisplatin also induced p53 in both cell lines, while docetaxel was studied only in MCF-7 cells, and was found to induce p53. Mdm2 was generally increased along with p53. AZT, however, had no clear effect on p53 or Mdm2 in either cell lines. In conclusion, because p53 was induced by three different DNA damaging agents in these models, but not with AZT, AZT probably did not cause DNA damage, although evidence of the carcinogenicity of AZT have been presented. Increase of Mdm2 indicates that the induced p53 is in functional form and that changes of Mdm2 are not the reason for p53 induction.

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TRP-1 AND TRP-2 MODULATE THE ACTIVITY OF POLY(ADP-RIbose) SYNTETASE IN VITRO

K. Ueda, M. Banasik, T. Stedeford, and D. L. Price. 1Institute for Chemical Research, Kyoto University. 1Kyoto, Japan. 1Institute of Environmental Engineering, Polish Academy of Sciences, Zabrze, Poland. 1Dept. of Environmental Management, Pinellas County Air Quality Planning and Analysis, Clearwater, FL.

Heterocyclic amines (HAs) are produced by pyrolysis of amino acids or proteins in fried, broiled, or grilled meats. These compounds are potent mutagens that increase rates of colon, mammary, and prostate tumors in rodent bioassays. The carcinogenic effect of these compounds is elicited from metabolic activation by the cytochrome P450 enzyme system to reactive metabolites(s). Interestingly, the P450-catalyzed cycle has the absolute requirement in vivo of NADPH. Here we report the effects of six groups of HAs on the activity of poly(ADP-ribose) synthetase (PARS). An enzyme implicated in several important biologic processes, such as DNA repair, apoptosis, or necrosis. PARS activation results in an immediate decrease in available NAD+ and may limit the activity of enzymes requiring this cofactor. Our results show that 3-amino-1, 4-dimethyl-5H-pyridine-4, 3-bjindole (Trp-P-1) causes a 91% and 94% decrease at 1 and 5 mM, respectively (IC50 = 0.22 mM). In contrast, 3-amino-1-methyl-5H-pyridine-4, 3-bjindole (Trp-P-2) at 1 mM causes a 134% increase in PARS activity vs. a 93% decrease in the activity at 5 mM (IC50 = 2.2 mM). In all other compounds tested, only an inhibitory effect was observed. The biological significance of these findings remains to be studied; however, it is possible that by inhibiting PARS, the available pool of NADH/NAD+ allows for increased activation of these compounds to toxic metabolites. (Supported by the Japan Society for the Promotion of Science)

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THE EFFECT OF ETHANOL, METHANOL, AND DIMETHYL SULFOXIDE ON POLY(ADP-RIbose) SYNTHETASE ACTIVITY.

M. Banasik, T. Stedeford, A. S. Pennal, and K. Ueda. 1Institute for Chemical Research, Kyoto University. 1Kyoto, Japan. 1Institute of Environmental Engineering, Polish Academy of Sciences, Zabrze, Poland. Departments of Epidemiology and Biostatistics, University of South Florida, Tampa, FL.

The inherent toxicity of a compound can be masked by the choice of solvent used to deliver the chemical of interest. The purpose of this study was to determine the effect of three organic solvents [ethanol, methanol, or dimethyl sulfoxide (DMSO) and water mixtures] at modulating the activity of poly(ADP-ribose) synthetase (PARS), a key enzyme in the repair of damaged DNA. A dose-response was performed with each of the relevant solvents. A 12% and 75% increase in activity above baseline was observed with 15% ethanol and 10% methanol, respectively. However, a near 20% decrease in activity was observed with 4% DMSO. Our results show an activating effect on PARs activity when ethanol or methanol is used at low concentrations, compared to the inhibitory effect of DMSO. PARS activity can deplete intracellular NAD+ and subsequently intracellular ATP; thereby causing necrotic cell death, if the capacity to replenish ATP is overcome. In contrast, apoptosis is associated with PARS cleavage and inhibition. Our data indicates that the interpretation of studies obtained when using common organic solvents may be dramatically skewed, either exaggerating the inherent toxicity of the compound or masking its potential for damage. (Supported by the Japan Society for the Promotion of Science)

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MECHANISM INVOLVED IN TOPOISOMERASE IIa INHIBITION BY PHOTOACTIVATED BAY Y3118.

C. E. Perrone and G. M. Williams. Department of Pathology, New York Medical College, Valhalla, NY.

Topoisomerase inhibition has been proposed as a possible mechanism involved in the photochemical toxicity and mutagenicity of photosensitizing antibiotics (FQs). We report here that some photchemically mutagenic FQs, but not all, inhibit the human topoisomerase II enzyme in vitro following UVA (365nm) irradiation. We studied Bay y3118 (y3118) and lormetrexol, which are highly photoactive, ciprofloxacin which is weakly photoactive and lomefloxacin, which is photosensitive. The degree of inhibition at 360 nM was as follows: Bay y3118, 15%; ciprofloxacin, 5%; lomefloxacin, 0% and maxifloxacin, 0%. The use of the UV-FQ-mediated inhibitory effects on the topoisomerase IIa was further examined using the photoinhibitory y3118. Topoisomerase IIa inhibition required the simultaneous presence of y3118, DNA and enzyme in the UVA irradiated reaction mixture indicating the possible formation of a ternary structure. UVA-FQ-mediated topoisomerase IIa inhibition also correlated with the increased binding of y3118 to DNA:topoisomerase complexes and was not prevented by the antioxidant TEMPO suggesting that reactive oxygen species were not involved in the inhibition. We conclude that some photchemically genotoxic FQs inhibit the human topoisomerase IIa (i.e. Bay y3118) possibly by UV-induced affinity of FQs to DNA:topoisomerase complexes, but that inhibition of topoisomerase IIa does not account for the photochemical activity of other FQs (i.e. lomefloxacin). Supported by the NIH Grant CA 86056.

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RESISTANCE TO SILICA-INDUCED LUNG FIBROSIS IN SENESCENT RATS: ROLE OF ALVEOLAR MACROPHAGES AND TUMOR NECROSIS FACTOR-ALPHA (TNF).

P. Cortini, A. Giani, S. Peano, M. Marinovich and C. L. Galli. Pharmacological Sciences, University of Milan, Milan, Italy.

PURPOSE: We have previously demonstrated in rats alveolar macrophages (AM), that aging was associated with a progressive decline in the ability of these cells to produce TNF. The purpose of the present study was to investigate the effect of aging on silica-induced lung toxicity, for which it has been demonstrated the requirement of TNF for the development of silica-induced pulmonary fibrosis.

SUMMARIZED DESCRIPTION: Sprague-Dawley rats 3 and 18 month old were intratracheally instilled with 30 mg of silica or saline as control. Two weeks following instillation, rats were killed by bronchoalveolar lavage fluid (BAL) parame ters (total protein, LDH, TNF and cellularity), total lung hydroxyproline, as measurement of collagen deposition, and lung histopathology. RESULTS: In young animals, as expected, silica induced a significant increase in BAL protein, TNF, LDH as well as in cell numbers, which correlate with increased collagen deposition and histologic nodular lesions formations. In old rats, however, no changes in BAL or lung parameters were observed following silica instillation. These in vivo results were also confirmed in vitro, where silica failed to induce TNF release in AM obtained from old animals. This defective response to silica could be explained with the defective
protein kinase C translocation, due to a reduction in its anchoring protein RACK-1 in old rats. Use of RACK-1 antisense oligonucleotide reduced the response of young macrophages to silica, supporting the idea that age-associated alterations in AM signal transduction pathways contribute to decreased sensitivity to silica-induced lung fibrosis.

373 THIOL ANTIOXIDANTS INHIBIT THE ADJUVANT EFFECTS OF AEROSOLIZED DIESEL EXHAUST PARTICLES IN A MURINE MODEL FOR OVALBUMIN SENSITIZATION.

M. J. Whitehead1, M. Zhang2, N. Li3, M. Howertz1, S. K. Nelson4, N. Brechun5, D. Diaz-Sanchez and A. Nel6. 1Pathology and Lab. Med., UCLA, Los Angeles, CA; 2Division of Critical Immunology and Allergy, UCLA, Los Angeles, CA; 3Division of Infectious Disease, UCLA, Los Angeles, CA; 4and Webb-Waring Antioxidant Research Institute, UCLA, Los Angeles, CA.

Although several epidemiological studies indicate a correlation between exposure to ambient particulate matter (PM) and adverse health effects in humans, there is still a fundamental lack of understanding of the mechanisms involved. We set out to test the hypothesis that reactive oxygen species are involved in the adjuvant effects of diesel exhaust particles (DEP) in a murine ovalbumin (OVA) sensitization model. First, we tested six different antioxidants, N-acetylcysteine (NAC), butylamine (BUC), thiamin, luteolin, trolox (vitamin E) and ascorbic acid, for their ability to interfere in DEP-mediated oxidative stress in vitro. Of the six agents tested only the thiol antioxidants, BUC and NAC, were effective at preventing a decrease in intracellular GSH/GSSG ratios, protecting cells from protein and thiol oxidation, and interfering with HO-1 expression. We therefore selected the thiol antioxidants for testing in the murine OVA inhalation exposure model. Our data demonstrate that NAC and BUC effectively inhibited the adjuvant effects of DEP in the induction of OVA-specific IgE and IgG1 production. Furthermore, NAC and BUC prevented the generation of lipid peroxidation and proteolytic oxidation in the lungs of OVA + DEP-exposed animals. These findings indicate that NAC and BUC are capable of preventing the adjuvant effects of DEP inhaled, and suggest that oxidative stress is a key mechanistic component in the adjuvant effect of DEP. Antioxidant treatment strategies may therefore serve to alleviate allergic inflammation and may provide a rational basis for treating the contribution of PM to asthmatic disease. (Supported by US Public Health Service Grant AI54567, EPA Southern California Particle Center, and by NIAID AI07126).

374 THIOL AND IMMUNE EXPOSURE DEPENDENT RESPONSE TO OVALBUMIN IN SENSITIZED RATS.

N. H. Al-Humaidi1,2, D. D. Sigel3, D. M. Lewis4, W. M. Burger5, J. Y. Ma6, D. N. Weissman7 and J. K. Ma1. 1ASB, NIOSH, Morgantown, WV; and 7School of Pharmacy, West Virginia University, Morgantown, WV.

Brown Norway rats were exposed 30 min to the antigen, ovalbumin (OVA) at 7.2, 68, 93, 111 or 156 mg/m3 on days 1, 8, 15, and 29. Tissue was collected one day after the last exposure. Cystine (CYSH), glutathione (GSH), and markers of inflammation in bronchoalveolar lavage fluid (BALF) were measured. Alveolar macrophage (AM) and pulmonary associated lymph nodes (PALN) were isolated and assessed for CYSH and GSH concentrations. Specific IgG and IgE, and lung tissue IFN-gamma, IL-4, and TNF-alpha mRNA levels (standardised with G3PDH) were the immunological endpoints studied. Rats were immunologically sensitized as evidenced by exposure related increases in all immunological parameters and OVA challenge induced increases in BALF albumin, protein and lactase dehydrogenase. In addition, OVA caused a modest elevation at low OVA challenges, but sharp reduction in AM thiols at higher OVA challenges. Concomitantly, OVA exposure dependent increases in BALF CYSH and GSH were observed. CYSH, but not GSH was elevated in PALN of OVA challenged rats. In summary, antigen exposure dose dependent alteration of thiol and immune parameters were demonstrated in OVA sensitized and challenged rats.

375 PROTEASE ENHANCEMENT OF IgG1 AND IgE ANTIBODY RESPONSE TO HEMICELLULASE IN MOUSE MODELS OF ALLERGY.


The mouse intranasal (MINT) and mouse aspiration (MappT) tests are being developed as replacements to the guinea pig intratracheal (GPIT) test used to assess the allergenic potential of protein allergens. Protease enzymes have been shown to enhance the IgG1 antibody response to non-protease enzymes in guinea pigs exposed to mixtures of protease and non-protease enzymes (Sarlto, et al. JACI, 1997, 100:480). The shift in the antibody response to enzyme mixtures in the GPIT test is used to adjust occupational exposure guidelines. To replace the GPIT test, we must show similar shifts in antibody responses to enzyme mixtures in the mouse models. BDF1 mice were exposed via intranasal (IN) instillation to protease, hemicellulase or a mixture of protease + hemicellulase on days 1, 3, 10, 17 and 24; sera were collected on day 29. Similarly, mice were exposed to the same enzymes via aspiration (ASP) instillation on days 1, 3, and 10; sera were collected on day 15. IgG1 and IgE antibody to each enzyme were measured by ELISA. IgE titers were randomly confirmed by the rat passive cutaneous anaphylaxis (PCA) test. The protease shifted the IgG1 antibody response to the hemicellulase - 3x in IN and ASP doses mice. The shift was dependent upon the amount of protease in the mixture - the lower the protease levels in the mixture, the smaller the shift. The protease also shifted the IgE antibody response to the hemicellulase in IN and ASP dosed mice. The enhancement was greater for IgE than for IgG1. The antibody response to protease was not altered by the presence of the hemicellulase. Testing of the protease + hemicellulase mixture in the GPIT test also showed a 3x to 4x shift in IgG1 antibody to the hemicellulase. Therefore, there is consistency between the guinea pig and mouse models. Continued work with mixtures of enzyme allergens is needed to develop the mouse model as a full replacement to the GPIT test.

376 RESPIRATORY PHYSIOLOGICAL RESPONSES TO AN EXTRACT OF Stachybotrys chartarum IN BALB/C MICE.

M. E. Vitanza, N. Haykal-Coates, S. H. Gavetti, M. J. Selgrade and M. D. Ward. 1AFR CVM, NCSU, Raleigh, NC and 2NHEERL ORD, USEPA, Research Triangle Park, NC.

Exposure to Stachybotrys chartarum has been associated with the development of serious health problems in humans known as "sick building syndrome". Previous studies by our group demonstrated respiratory exposure to a pool of S. chartarum extracts caused biochemical and immunological responses indicative of a respiratory allergic response. The goal of this study was to assess the ability of this extract to cause alterations in respiratory physiological responses similar to those observed in human allergic asthma. Five isolates of S. chartarum obtained from the in vivo airway-damaged houses were grown and combined in approximately equal weight amounts, extracted using Hanks Balanced Salt Solution (HBSS) + Tween-80, and filtered sterilized to form a crude antigen preparation (SCPE-1). Female BALB/C mice were anesthetized and exposed to 4 aspirations of 50 μl volume containing 10 mg of SCPE-1, BSA as a non-allergic protein, or HBSS over a 4-week period. Barometric whole body plethysmography was performed to measure enhanced pause (PenH) 10 minutes prior to (baseline) and 1 hour following each aspiration exposure to assess immediate respiratory responses. Additionally, airway hyperresponsiveness to nebulized methacholine (MCh) was assessed on days 1 and 4 following the 4th aspiration exposure. Exposure to HBSS or BSA did not alter baseline PenH values, PenH following the aspiration exposures, or airway responsiveness to MCh. Exposure to SCPE-1 resulted in a 4.7-fold increase in PenH over baseline after the 3rd exposure, increasing to 5.6-fold after the final exposure, and increased responsiveness to a 32 mg/ml MCh aerosol challenge. We conclude respiratory SCPE-1 exposure causes respiratory physiological responses similar to those observed in human allergic asthma. However, BSA does not generate the respiratory physiological responses expected of an allergic protein when administered by aspiration. (Supported by NCSU/EPA Cooperative Training Agreement CR826512010). (This abstract does not reflect EPA policy).
in these animals were 4-fold the level of control 24 hours after exposure and persisted at that level by 96 hours after exposure. No pulmonary lesions were found in mice of any group. TDI-exposed mice did have an acute rhinitis with associated epithelial necrosis and exfoliation. This study is the first to demonstrate that intranasal administration of LMWCG is an effective method of sensitization and results in an upregulation of a critical mediator of allergic airway disease, IL-4, within the lung after challenge (Supported in part by American Chemistry Council Grant # 0051).

378 CHARACTERIZATION OF IGE INDUCING PROTEINS FROM EXTRACTS OF METARHIZIUM ANISIOPIAE

We have previously demonstrated BALB/c mice exposed to Metarhizium anisioiapi crude antigen (MACA) develop immune and pulmonary responses typical of allergic and asthma. Soluble factors from the fungal components (mycelium, conidia, and inducible proteases and chitinases) were combined in equal protein amounts to form the crude antigen. The fungal allergens were identified by SDS-PAGE/western blot analysis using IgE containing hyper-immune mouse serum as the source of primary antibody and traditional chromogenic enzyme substrate for detection. India ink staining detected more than 50 proteins in MACA. Of these 4-5 induce an IgE response and comprise approximately 12-15% of the MACA proteins by densitometric analysis of the India ink-stained, framed blot. When the MACA component extracts were examined individually, we determined that 4 of these proteins were detectable in the mycelium extract, and 2 were detectable in the inducible extract (1 homologous by MW to a mycelium protein identified and 1 unique protein). However, there were no IgE inducing proteins detected in the conidia extract.

We have begun to re-assess these extracts using a chemiluminescence detection system that is more sensitive. The IgE inducing proteins in the mycelium extract identified as met a 1, met a II, met a III, and met a IV have apparent molecular weights of 127.6 kDa, 114.7 kDa, 89.7 kDa, and 52.6 kDa, respectively. Furthermore, we have determined the isoelectric points of the allergens proteins by isoelectric focusing (IEF) and confirmed the apparent molecular weights by two-dimensional gel analysis. Using SDS-PAGE western blot analysis we have also found batch-to-batch variability in our fungal extracts. Although we have found these are apparent changes in the concentration of the allergenic proteins in different extract batches, there do not appear to be differences in the proteins detected. (This abstract does not reflect EPA policy)

379 EFFECT OF ORGANIC PARTICLE EXPOSURE ON ALLERGIC AIRWAY DISEASE IN MICE.

Past studies have documented the adjuvant effect of diesel exhaust and carbon black (CB) particles on allergic airway disease (AAD) in mice immunized to elicit a TH2 lymphocyte phenotype. However, few studies have examined the effect of particle exposure on reversing the normal hyporesponsive state in animals immunized to elicit a TH1 lymphocyte phenotype. To investigate if CB exposure will alter the hyporesponsive TH1 phenotype to a responsive TH2 phenotype, mice were exposed to CB by intranasal (IN) instillation during the challenge phase of ovalbumin (OVA) induced AAD. Mice were immunized with either OVA-ALUM (TH2 phenotype) or OVA-CPA (TH1 phenotype) and then challenged with OVA aerosol (1 hr/day) for 3 days. Mice received 200µg CB IN 4 hrs prior to each OVA aerosol challenge. Animals were killed 24 hrs later and evaluated for BAL cell number and type. Mice expressing a TH1 response to OVA demonstrated significantly fewer eosinophils than mice expressing a TH2 phenotype (50% versus 87%) confirming the hyporesponsive state. Contrary to expectations, administration of CB to TH1 mice decreased total BAL cell number with no change in % eosinophils (50% versus 51%). Administration of CB to TH2 mice also decreased total cell number and proportion of eosinophils from 87% to 49%. These data suggest that CB exposure decreases the pulmonary allergic response in both TH1 and TH2 phenotype mice. (Funded by ES NIHH04933)

380 OXAZOLONE-INDUCED DIRECT PLNA REACTIONS ARE SUPPRESSED IN MICE ORALLY PRETREATED WITH OXAZOLONE.

There is a need for preclinical assays that can identify low molecular weight compounds (LMWCS) with the potential to cause systemic hypersensitivity (SH). One such assay being examined is the rodent popliteal lymph node assay (PLNA). Based on >80 LMWCS, in general, only LMWCS associated with SH cause PLN reactions in rodents. However, despite this preliminary success, there are fundamental questions that remain unanswered. One such question is concerned with false positives. For example, it is hypothesized that contact sensitizers (CS) will produce strong PLN reactions but are not expected to cause SH following oral administration due to oral tolerance. The objective of this study was to examine the immunomodulating potential of the CS oxazolone (OX) and DNCB in the BALB/c PLNA, and to examine what effect oral pretreatment with OX had on the reactions. In naïve mice, both CSs induced strong PLN reactions. OX induced dose-dependent increases in PLN weight, cellularity, the % of CD4+ T cells, and the % of B cells, with a consistent decrease in the % of CD8+ T cells. Next, the PLNA was conducted in mice gavaged 3 times with OX. Mice gavaged with OX had suppressed PLN reactions following the injection of OX (decreased PLN size, weight and cellularity), as well as a decrease in the % of CD4+ helper T cells. Oral treatment with OX had no observable effect on the PLN reaction induced by DNCB. Finally, adaptive transfer studies were conducted to examine the mechanism of PLN unresponsiveness. It was found that unfractonated splenocytes isolated from mice gavaged with OX adoptively transferred (iv) PLN unresponsiveness to naïve mice. Collectively, these observations demonstrate that OX is a potent immunomodulator in the PLNA.

381 CHARACTERIZATION OF THE SENSITIZING POTENTIAL OF A STRONG DERMAL IRRITANT USING AN ENHANCED LOCAL LYMPH NODE ASSAY.

Strong irritants can give ambiguous results in animal models used for predicting dermal sensitization potential. An enhanced LLNA protocol was devised to better characterize the sensitization potential of a long chain alkylamine (LCAA), a strong dermal irritant that had previously given conflicting results in other sensitization assays. In this study, groups of five CBA mice were treated by topical application of LCAA (0.1, 0.3, 1.0% w/v) once daily to the dorsum of each ear for three consecutive days. Additional groups were treated with the known sensitizers DNCB (0.25% w/v) or HCA (50% w/v), or the non-sensitizing irritant SLS (25% w/v). Five days after the initial dose, the mice were injected (i.p.) with 5-brorno-2-deoxyuridine (BrdU) to label proliferating cells. Auricular lymph node vessels were isolated and the number of BrdU+ cells was determined for individual animals by flow cytometry. Immunophenotype analysis of the nodal cells was conducted using the marker combinations B220/CD3 and IA/CD69 to determine the B/T cell ratio and the activation state of the nodal lymphocytes respectively. Ear thickness was also evaluated for all animals. LCAA caused a significant increase in ear thickness and a dose-dependent increase in lymph node cell proliferation with a maximum Stimulation Index (SI) of 125.9 (EC3 = 0.1%), while the known sensitizers DNCB and HCA gave a SI of 104.6 and 30.1 respectively. Both positive control substances and higher dose levels of LCAA also caused a significant increase in the B/T cell ratio (B220/CD3+) and IA/CD69+ cells with SLS causing a significant increase in vessel swelling and an SI of 3.2 but no increase in cellular markers. Despite the significant irritant response induced by LCAA, the magnitude of the proliferative response and the activation state of cells localized in the nodes of treated mice identify LCAA as a potential dermal sensitizer.

382 RELEVANCE OF HUMAN CYPSO450 1A1 AND 1B1 IN THE METABOLISM OF FRAGRANCES.
B. Blomcke, A. Helme, C. Skazik and H. F. Merk. Dermatology, University Hospital, Aachen, Germany.

Fragrances are widely encountered in our daily environment and are known to be a common cause of allergic contact dermatitis. Such small molecular compounds usually need binding to high molecular weight compounds in order to become antigenic. The knowledge about the metabolic activation is still limited. Recently, we found that the proliferative responses of lymphocytes from fragrance-allergic patients were increased in the presence of human liver microsomes or recombinant CYP1A1 (Sieben et al., 2001). In addition, we found that eugenol but not isoeugenol produced a 20-fold increase in CYP1A1 steady state mRNA levels in human primary keratinocytes (Al Muaoodi et al., 2001). With the aim of providing further insights into the mode of action of eugenol (4-propenyl-2-methoxyphenol) and the structurally related compound isoeugenol (4-propenyl-2-methoxyphenol) in human skin cells, we studied whether CYP1B1, AhR and ARNT are also involved in the activation of these compounds. We found, that eugenol additionally produced a 4-fold increase in CYP1B1 steady state mRNA levels but no induction
Eighteen chemicals, known to induce allergic contact dermatitis in man, including various thiuram, carbamates, and benzothiazole compounds and one antine were tested. The EC50 (effective concentration inducing 50% of the proliferation of lymph node cells (stimulation Index, SI = 1.5)) was calculated with non-linear regression analysis, including a bootstrap method for determination of the 95%-95% confidence interval of the EC50 value. The compounds tested were thiuram TMTM (tetrathiuram monosulphide), TMTT (tetrathiuram disulphide), TCTMT (tetrathiuram trisulphide), TCTTT (tetra[1-mercaptobenzimidazole], and TPT (disodium ethylenedithiothiocarbamate), 1,2,4-benzothiazole-3-(2H)-thione, 2-benzothiazolecarboxamide, and DEA (diethylenetriamine). This procedure identified 14 out of the 15 chemicals tested as sensitive, while for one chemical, TDB, no EC50 could be calculated due to low responses and a lack of a Dose-Response relationship in the data obtained. The ranking order of the chemicals with increasing EC50 values (and thus decreasing allergenic potency) was found to be: ZDEC, TMTT, TMTF, TEP, ZPC, ZDCC, MTS, TPT, TMT, MBT, PT, ZMBT, ZTBD, DEA, and ZDBZ. Our results indicate that the chemicals of choice for use in latex production would be for the thiuram compounds TBTB, for the carbamates ZDBZ and for the benzothiazoles ZMBT. However, one has to be aware that besides potency also the total amount of residual chemical present in the final product is important for allergy induction.


Recently the presence of antipolymer antibodies (APA) has been reported to be correlated with the disease status in silicone breast implant (SBI) recipients and patients with breast malignancy. A cross sectional study was conducted in a population of Dutch women with SBI and self reported severe complaints. SBI recipients (n=42) were clinically examined and blood samples were obtained. In the APA assay the binding of immunoglobulins to an acrylamide polymer-coated strip (2) was demonstrated after incubation with secondary antibodies and visualization by a colorimetric method. In 12 of 42 SBI recipients an increase in the level of polymer binding immunoglobulins was detected compared to a negative reference sample. 3 of these 12 showing a positive and 9 a weakly positive response. In 5 out of 12 non-SBI recipients including, for control on the performance of the APA assay, an increased level of polymer binding immunoglobulins was demonstrated, 2 of these 3 showing a positive and 1 a weakly positive response. In the general population in 5 out of 80 women a positive APA was found, and one of 80 showed a weakly positive response. The study population of SBI recipients was categorised in severity subgroups (as defined, advanced) based on the functional capacity and the physicians general assessment of pain and disease activity. Most (34 of 42) SBI recipients belonged to the limited severity subgroup. In conclusion, we did not find a high prevalence of polymer binding immunoglobulins (APA) in a group of Dutch SBI recipients with self reported severe complaints and a present biopsy showing the self-reported severe reaction and the observed mild clinical symptoms. No SBI exposure (mean 17 years) did not result in induction of polymer binding immunoglobulins in this minimal symptomatic study group. Antigen strips for the APA assay were kindly provided by Dr R.B. Wilson, Autoimmune Technologies, L.C.C., New Orleans, LA, USA.

387 EXPOSURE TO SINGLE OR TERTIARY MIXTURES OF N.N-DIETHYL-M-TOLUAMIDE (DEET). PYRIDOSTIGMINE BROMIDE (PYR), JP-8 JET FUEL AND STRESS IN AUTOIMMUNE PRONE MICE. G. S. Gilkeson, M. M. Peden-Atamia, J. G. Ed-Daly, S. Dabra, A. Ed-Daly, R. Hayes, L. Heschman, J. Miller, J. Smyth and D. Keil, Medical University of SC, Charleston, SC. Exposures to chemicals such as DEET, PYR, and JP-8 jet fuel (JP-8) are common in military environments, as well as in some occupations. During the Gulf War, military service personnel were exposed to DEET, PYR, DEET, and stress. Previous studies determined the lowest adverse effect level of immunological parameters to be 15.5 mg/kg DEET, 2 mg/kg PYR, 500 mg/kg JP-8, and 20 minutes exercise stress for 14 days. To investigate the contribution of these agents in accelerating or exacerbating autoimmune disease, a strain of autoimmune prone mice, MRL/lpr/lpr, were exposed for 14 days at age 12 weeks. At 16 weeks of age, immunological parameters in mice were assessed. No remarkable changes occurred in complete...
peripheral blood counts, spleen and thymus total cellularity and CD4/8 flow cytometry, except for a decrease in thymic CD4+CD8+ cells after exposure to J-P 8 only. Furthermore, macrophage nitrite production or splenic lymphocyte proliferation after exposure to concanavalin A or lipopolysaccharide was not significantly different. Proteinuria was also assessed in these mice at 14, 16 and 18 weeks. At 18 weeks of age, MRL-lpr/lpr female mice had an expected time-dependent increase in proteinuria. Furthermore, single exposure to either PYR or exercise stress, an increase in proteinuria was noted over controls. Single exposure to DEET or J-P 8 minimally affected the development of proteinuria as compared to controls. Furthermore, mice exposed to the stress mixture (20 min: PYR + 15.5 mg/kg DEET + 2 mg/kg PYR) or J-P 8 mixture (500 mg/kg J-P 8 + 15.5 mg/kg DEET + 2 mg/kg PYR) demonstrated increased proteinuria over control values, but this change was not statistically greater than levels achieved after single exposure to PYR or stress. Thus far, these data suggest that several immunological endpoints were not remarkably exacerbated in an autoimmune mouse model; however, proteinuria may be accelerated.

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THE POSSIBLE ROLE OF THE GUT IN D-PENICILLAMINE-INDUCED AUTOIMMUNITY IN THE BROWN NORWAY (BN) RAT.

M. Masson and J. P. Leech. Pharmacological Sciences, University of Toronto, Toronto, ON, Canada.

In male Brown Norway rats, high dose D-penicillamine (D-Pen; 20mg/day) induces an autoimmune syndrome in 70% of animals within 6 to 8 weeks that is similar to drug-induced lupus in humans. The gut is believed to be central to the induction of oral tolerance. Low doses induce tolerance, while high doses induce autoimmunity. In the Brown Norway model, a tolerance-producing regimen consisting of a two-week period of low dose D-Pen (5mg/day) followed by high dose D-Pen results in none of the animals developing lupus. The hypothesis to be tested is that gut immunomodulation by high dose D-Pen treatment could provoke the development of autoimmune disease. Male Brown Norway rats (n=3) were given 20 mg/day of D-Pen for periods of 0, 48 hr and 1 week. Immunohistochemistry was performed on frozen sections of the cecum and the large intestine to examine the expression of ED1+ macrophages and B7.2, a major costimulatory molecule involved in initiating immune responses. These early time periods were chosen as changes in the distribution of ED1+ and B7.2+ cells will reflect changes in response to the drug, rather than simply reflecting signs of disease. In the cecum, both ED1+ and B7.2+ cells increased after only 48 hours of exposure to high dose D-Pen. By one week, large areas of infiltration were apparent in all sections. In the large intestine, similar findings are apparent. Interestingly, exposure to D-Pen results in the expression of B7.2+ by epithelial cells. These data indicate a possible role of the gut in initiating D-Pen-induced autoimmunity.

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PENCILLAMINE INDUCED LUPUS-LIKE SYNDROME: IN VIVO.

B. Seguin, C. Ju, L. Pohl and J. Urech. Pharmacological Sciences, University of Toronto, Toronto, ON, Canada.

BACKGROUND: Idiosyncratic drug reactions are often explained by the Hapten Hypothesis, i.e. covalent binding of a drug or its metabolite to proteins leads to an immune response. We used an animal model, penicillamine-induced autoimmunity in the Brown Norway rat, to study the relationship between covalent binding and the mechanism of an idiosyncratic reaction. METHODS: Antibodies against penicillamine were induced in rabbits by injecting a penicillamine-RLH antigen. Using ELISA, the rabbit serum showed specificity for penicillamine at dilutions as high as 1:1000. RESULTS: Male Brown Norway rats were given penicillamine (20 mg/day in water). Within animals developed evidence of autoimmunity, the animals were killed and the spleen, liver and skin tissues were analysed by immunohistochemistry and western blotting. Immunohistochemistry analysis revealed low binding in spleen and liver of sick rats, but much higher binding was observed in collagen fibers of the skin. Western blot analysis under reducing conditions revealed specific binding, but under non-reducing conditions, we identified several protein species that were covalently bound by penicillamine. We identified that the binding involved a disulfide linkage. The molecular weight of the proteins bound by penicillamine ranged between 48 and 94 KDa. Attempts at finding autoantibodies against collagen were unsuccessful because of high background binding of the rat serum. DISCUSSION: It has been reported that penicillamine can bind aldehydes on collagen and cause fragility of bones, skin and delay wound healing. Due to the chemical structure of penicillamine, we expected that binding would occur with all sulfhydryl-containing proteins and not just collagen. We suspect that penicillamine is being removed enzymatically or non-enzymatically. The high degree of binding to collagen may be because enzymes that catalyse an exchange are mostly intra-cellular. The role of binding of penicillamine to collagen in the pathogenesis of this idiosyncratic reaction is currently under investigation.

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EVALUATION OF AN ENVIRONMENTAL CONTAMINANT, CADMIUM, ADMINISTERED IN DRINKING WATER IN THREE AUTOIMMUNE MODELS.

E. K. Leffel and K. L. White, Jr. Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

The etiology of autoimmune disease is largely unknown. However, there is evidence that there are genetic factors that predispose some individuals. When these people are assaulted with environmental triggers such as drugs, viruses, or chemicals, then autoimmune disease can result. The purpose of these studies was to determine if cadmium (Cd) was a potential candidate for producing autoimmune disease (AD) in genetically predisposed individuals. NZB/W and MRL/lpr mice (autoimmunity models) and Brown Norway (BN) rat (autoimmunity model) were utilized. NZB/W: Mice were exposed to Cd in the drinking water at concentrations of 0 to 10, 000 ppb for 31 weeks. At 15 weeks of exposure, the Cd treated groups showed onset of AD, i.e. proteinuria, compared to 27 weeks for tap water control. Anti-nuclear antibody (ANA) levels were increased after 31 weeks of exposure in the 30 and 300 ppb groups. Splenocytes cultured from mice in the long-term study showed increased in IgG2a & IgG3 antibodies. In the serum, a similar trend was observed. MRL/lpr: Mice were exposed to 0 to 10, 000 ppb Cd for 11 weeks. Cd-treated groups developed proteinuria 3 weeks earlier than the vehicle group and a larger percentage developed ANA. Deposition of immune complexes in the glomeruli was confirmed by electron microscopy. BN: Rats were exposed to 0 to 300, 000 ppb Cd for nine weeks. Increases in the number of rats positive for ANA were observed in the Cd dose groups. The level of urinary Cd was significantly increased in the high dose group; notably, the level is 500-times lower than that required to produce renal lesions from Cd. Based on the development of ANA, and the low levels of Cd in the urine, Cd may be capable of inducing autoimmunity in the BN. The findings from these rodent models suggest low levels of Cd in drinking water have the potential to exacerbate AD. Supported in part by NIEHS contract 05455.

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MERCURY ACCELERATES AUTOIMMUNE DISEASE IN THE GRAFT VERSUS HOST DISEASE (GVHD) MODEL.

E. K. Siibergedt1, D. Hoover2, P. Nguyen1, L. Silva1, M. Lustberg1 and C. Vila1.

1Dept Epileptology, University of Maryland Med. Sch, Baltimore, MD, 2Dept of Neurobiology, University of Maryland Med. Sch, Baltimore, MD, 3Dept of Medicine, University of Maryland Med. Sch, Baltimore, MD and 4ICBM, University of Porto, Porto, Portugal.

Mercury (Hg) is immunotoxic in many species, and has been shown to elicit immunological and physiological effects similar to lupus, an autoimmune disease, in inbred rodent strains. We have utilized GVHD, a well-characterized model for lupus-like autoimmune disease, in which lymphocytes from a DBA/2 parent are injected into a C57Bl6xDBA/2-F, offspring host. This model, an alloantigen driven in vivo anti-host response, permits evaluation of Th1 and Th2 mediated responses in the development of disease. In this study, both donor and recipient mice were pretreated on alternate days for 3 weeks, by s.c. injection of HgCl2, at either 20 or 200 mg/kg. This treatment does not affect body weight or survival, but has been shown to alter Th1/Th2 signaling without affecting splenocyte subsets. Within 5 days after the last Hg treatment, lymphocyte transfer was done and disease then assessed by monitoring proteinuria, antibody levels, and mortality. Four groups of female mice were studied: control C57Bl6xDBA/2-F, animals; GVHD animals; GVHD+20 mg/kg Hg; and GVHD+200 mg/kg Hg. Mercury pretreatment significantly increased risks of early mortality in the GVHD model. At 4 months after disease initiation, 4 Hg animals had died (2 in the high group, followed by 2 in the low group) as compared to none in either control group. Also, Hg treatment significantly increased the likelihood of early and more severe proteinuria as compared to the controls or the untreated GVHD group. No changes were observed after 8 weeks in serum autoantibody (anti-sDNA IgG) levels; however further analyses will be conducted to measure specific autoantibodies. The results indicate that Hg may accelerate or worsen clinical autoimmune disease in susceptible populations. Support from the Levine Family Foundation and the Maryland Chapter of the Arthritis Foundation.

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SILICA ACCELERATED SYSTEMIC AUTOIMMUNE DISEASE IN LUPUS PRONE NEW ZEALAND MIXED MICE.

J. M. Brown, L. C. Pfau and A. Holian. Department of Pharmaceutical Sciences/SPHHS/Center For Environmental Health Sciences, The University of Montana, Missoula, MT.

The genetic backgrounds of lupus-prone murine models are valuable resources for studying the influence of environmental exposure on autoimmune diseases in sensitive populations. Genetic predisposition may be a prerequisite for the develop-
ment of spontaneous autoimmune disease, such as systemic lupus erythematosus (SLE), but the incomplete concordance of SLE in twins suggests that exogenous or environmental factors are also important. Epidemiological studies have shown associations between silica exposure and several autoimmune diseases, including scleroderma and ANCA-associated small-vessel vasculitis. To determine whether long-term silica exposure exacerbates systemic autoimmunity in genetically predisposed animals, New Zealand mixed mice were intranasally instilled with saline or saline suspensions of 1 mg silica. Biomarkers of autoimmune disease were measured every two weeks by ELISA. These included circulating immune complexes, autoantibodies to nuclear antigens (ANA), histone, and double stranded DNA. ANA levels measured by ELISA were significantly higher in silica treated animals compared to saline treated animals (0.444 ± 0.17 vs. 0.293 ± 0.03, p<0.05) 10 weeks post-exposure. Autoantibodies to histone were also significantly elevated after 10 weeks in silica treated animals compared to saline treated animals (0.587 ± 0.24 vs. 0.334 ± 0.045 p<0.05). Autoantibodies to dsDNA were significantly elevated after 8 weeks in silica treated animals compared to saline treated animals (0.117 ± 0.305 vs. 0.593 ± 0.231 p<0.05). Circulating immune complexes were seen in 6 of 8 silica treated mice, while none were seen in the saline treated mice at 10 weeks following exposure. Therefore, although NZM mice are susceptible to SLE, silica exposure significantly elevated levels of autoantibodies to nuclear antigens, including histone and dsDNA, as well as increasing circulating immune complexes, suggesting an induction/exacerbation of the course of this disease. This work was supported by NIH grant ES-04804.

393 ASCORBIC ACID AND GLUTATHIONE REDUCE 2-AMINO-4, 5-DICHLOROPHENOL (2A45CP) TOXICITY IN RENAL, CORTICAL SLICES FROM MALF FISCHER 344 RATS.

M. A. Valentinovic, J. G. Ball, H. Sun and G. O. Rankin, Dept of Pharmacology, Marshall University School of Medicine, Huntington, WV.

2A45CP is an aromatic ring hydroxylated metabolite of 3, 4-dichloroaniline. 3, 4-Dichloroaniline is nephrotoxic with primary damage located within the proximal tubules. This study assessed the in vitro toxicity of 2A45CP in renal cortical slices and second, determined the effect of antioxidants and sulfhydryl containing agents on the severity of 2A45CP toxicity. Renal tissue was isolated from male Fischer 344 rats (150-220 g). Renal slices were rinsed 3 times for 3 min each in 3-mL renal Krebs buffer. Tissues were incubated for 90-120 min with 0-1 mL 2A45CP in a separate series of experiments, the slices (50-100 mg) were preincubated for 30 min with 1 mM dithiothreitol (DTT), glutathione (1 mM) or 2 mM ascorbic acid to exposure to 0, 0.05, 0.1 or 0.25 mM 2A45CP. 2A45CP produced a concentration and time dependent increase in LDH leakage from renal cortical slices. Total glutathione levels were diminished by 0.5 mM 2A45CP within 60 min. Renal slices incubated for 60 and 120 min with 0.05 and 0.1 mM 2A45CP had lower malondialdehyde levels than control. Pretreatment with DTT did not alter 2A45CP toxicity. 2A45CP toxicity was reduced by pretreatment with GSH and ascorbic acid. These findings indicate that 2A45CP is directly toxic to renal cortical slices and that cytoxicity is at least partially mediated by a reactive intermediate. (Supported by NIH Grant ES68834).

394 NEPHROTOXIC EFFECTS OF THE R- AND S- ENANTIOMERS OF N-(3, 5-DICHLOROPHENYL)-2-HYDROXYSSUCINIMIDE (NDHS) AND ITS SULFATE CONJUGATE.


The agricultural fungicide N-(3, 5-dichlorophenyl)ssucinimide (NDHS) induces nephrotoxicity characterized by polyuric renal failure and proximal tubular necrosis. Recent findings suggest that NDHS nephrotoxicity is mediated, at least in part, through sulfate conjugates of oxidative NDHS metabolites. We have also shown that the stereoisomerism of NDHS metabolites may also be an important determinant for NDHS nephrotoxicity. The purpose of this study was to determine if stereoisomerism is a factor in the nephrotoxicity induced by NDHS metabolite N-(3, 5-dichlorophenyl)-2-hydroxyssucinimide (NDHS) in vivo or the O-sulfate conjugate of NDHS (NSC in vivo). Male Fischer 344 rat (4-6 groups) were administered R- or S- NDHS (0.05, 0.1 or 0.2 mmol/kg) or vehicle ip. Renal function was then monitored for 48 hr. NDHS (0.1 or 0.2 mmol/kg) had little effect on renal function, with only minor changes in glucosuria, proteinuria and elevated blood urea nitrogen (BUN) noted at the higher dose level. In contrast, S-NDHS induced minor renal effects at 0.05 mmol/kg, but marked nephrotoxicity at 0.1 mmol/kg. The nephrotoxic potential of R- and S-NSC (0.5, 0.75 or 1.0 mmol/L) was determined using freshly isolated renal cortical cells (4 million cells/ml) from untreated male Fischer 344 rats. Cytoxicity was determined by measuring lactate dehydrogenase (LDH) release at the end of a 60-min incubation. R- and S-NSC-induced LDH release was similar at all concentrations tested, with R-NSC increasing LDH release slightly more than S-NSC at the 1.0 mmol/L. These results support the conclusion that stereoisomerism is a factor for NDHS metabolite nephrotoxicity and that the role of stereoisomerism occurs outside the kidney for nephrotoxic sulfate conjugates. Supported by NIH grant DK31210.

395 METALLOTHIONEIN ISOFORM 3 AND PROXIMAL TUBULE VECTORSOR ACTIVITY, TRANSPORT.

D. kim1, S. H. Garrett2, M. A. Sene3, S. Somji4 and D.A. Sene4. 1Univ. West Virginia University, Morgantown, WV and 2Pathology, West Virginia University, Morgantown, WV.

Metallothionein isoform 3 (MT-3) has been shown to be expressed in the proximal tubule cells of the human kidney. The goal of the present study was to further characterize the basal expression of MT-3 in the proximal tubule and to determine if MT-3 participates in the maintenance of proximal tubule cell function. Expression of MT-3 mRNA was determined in the intact proximal tubule using microdissection and RT-PCR. MT-3 mRNA was detected in the proximal tubule of the in situ kidney with relative expression in excess to that of the beta-actin housekeeping gene. The normal HPT cells were shown to express both MT-3 mRNA and protein and to form domes, while the immortal proximal tubule cell line HK-2, was shown to have no expression of MT-3 mRNA and protein and did not form domes. The stable transfection of MT-3 in the HK-2 cells restored MT-3 expression and dome formation in these cells. These results demonstrate that MT-3 mRNA is present in the human proximal tubule and suggest that MT-3 expression is involved in the transmembrane function of a renal cell line that retains properties of the proximal tubule.

396 DIFFERENT EFFECTS OF OCHRATOXIN A AND B ON ENZYME ACTIVITY IN VITRO.

D. R. Dietrich1, M. E. Stack2 and K. Steenstra1. 1Environmental Toxicology, University of Konstanz, Konstanz, Germany and 2USFDA, Washington, DC.

Ochratoxin A is a toxic secondary metabolite produced by several Aspergillus and Penicillium species. It has been reported to be primarily nephrotoxic and causally related to urinary tract cancers. Effects in the liver have also been reported. Several studies have shown the influence of OTA on hepatic glycogen levels but with contradicting results. On the one hand OTA has been demonstrated to cause hyperglycemia in vivo, while on the other hand, other groups have observed decreased activity of glycogenolytic enzymes in vivo following OTA exposure. The glycogen content of the kidney is suggested to play an important role in the provision of energy, especially under the anaerobic conditions of the inner medulla. Thus, an influence of OTA on renal glycogen levels could be involved in its nephrotoxic action. Nothing is known about the influence of the less toxic analog, OTB on either renal or hepatic carbohydrate metabolism. As the muscle phosphorylase is predominant in the rat kidney and also dominant in the kidneys of several other species, the influence of OTA on this isozyme was studied. OTA and OTB were covalently coupled to horseradish peroxidase (HRP) and used similarly to an antibody for dot blot analysis of glycogen phosphorylase binding. Enzyme-binding was observed for both substances. Phosphorylase itself cannot be measured directly thus, activity was measured using a combined optical test with glucose-6-phosphate dehydrogenase (Glu-6-PDH) as indicator enzyme. Inhibition of the enzyme reaction was observed only with high (>75μM) OTA concentrations. This "inhibition" was however, actually due to inhibition of the indicator enzyme Glu-6-PDH. In contrast, Glu-6-PDH was not inhibited by OTA. The findings of this study yield no clear evidence of a specific influence of OTA on kidney carbohydrate metabolism but suggested OTA to be able to influence enzyme activity in general. Moreover they support the strict structural requirements for OTA and OTB activity, implying either a weaker binding to, or faster dissociation of OTB from the enzyme.

397 SEX- AND AGE-SPECIFIC EFFECTS OF OCHRATOXIN A IN PRIMARY HUMAN KIDNEY CELLS (HKC).

P. Wolf1, E. O’Brien1, A. H. Heusser2, M. E. Stack3, R. Thiel1 and D. R. Dietrich4. 1Environmental Toxicology, University of Konstanz, Konstanz, Germany, 2USFDA, Washington, DC, 3Urology, Klinikum Konstanz, Konstanz, Germany and 4EUREGIO Eutocology Laboratory, EESL, Konstanz, Germany.

OTA has been demonstrated to be nephrotoxic and carcinogenic in rodents and nephrotoxic in pigs, with distinct species- and sex-differences in susceptibility. Chronic dietary intake of ochratoxin A (OTA) via contaminated food, leading to serum concentrations in the nM range, has also been associated with increased incidences of urothelial tumors (UT) and nephropathy in humans (e.g., Balkan Endemic Nephropathy, BEN). Epidemiological studies of the regions endemic for
BEN and UT have suggested that women are slightly more affected than men. Previous results in our laboratory have indicated OTA to disrupt the cell cycle of primary porcine renal epithelial cells. Therefore, to investigate the mechanism(s) of OTA-induced nephropathy and cancer in humans, the antiproliferative and cell cycle effects of exposure to OTA (18 and 48 hrs) were investigated in primary human male and female renal epithelial cells (HREC). To assess sex-related differences in sensitivity to OTA, cells from both sexes and from several age groups were investigated. Average donor ages were 65.8 ± 9.6 (n=4) and 62.5 ± 13.6 (n=4) for male and female, respectively. A further sample was obtained from a 14-year-old male donor. Following cell preparation from biopsy material and characterization for epithelial origin, the antiproliferative effects of OTA were determined using a standard nuclear counting procedure (proliferating index). First statistically significant proliferative arrest was found after 48 hrs at concentrations as low as 10 nM (female) and 50 nM (male) OTA. HREC obtained from the 14-year-old boy were more sensitive than those from all other male donors, showing first effects at 10 nM OTA. These results imply female HREC to be slightly more sensitive to OTA than the male counterparts and furthermore, suggest that children may be more susceptible to the toxic effects of OTA.

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CYTOTOXICITY OF OTA AND OTB IN HUMAN AND PORCINE RENAL CELLS AFTER REPEATED EXPOSURE.


Environmental Toxicology, University of Konstanz, Konstanz, Germany; EUREGIO Ecotoxicology Laboratory, EESL, Konstanz, Germany; USFDA, Washington, DC; and Urology, Klinikum Konstanz, Konstanz, Germany.

Ochratoxin A and B are known contaminants in foodstuffs and suspected of being one of the etiological agents responsible for Balkan endemic nephropathy (BEN) and urinary tract tumors. The dechloro-analogue, OTB, is less toxic than OTA in animal studies, suggesting specific structure-activity requirements for toxicity. Ochratoxin A has previously been shown to bind to serum albumin and is also suspected to bind to cellular proteins of the kidney, which could be a prerequisite for toxicity. To detect these renal proteins, OTA and OTB were coupled covalently to horseradish peroxidase (HRP) and were used similarly to an antibody in Western blot analysis. With this method it was investigated if variations in OTA and OTB binding to renal proteins could explain the observed structure-specific and sex-differences. Several proteins from rat, pig and human kidney as well as from proximal human kidney cells (HREC) were detected using OTA-HRP as staining method and displayed large intensity differences. Comparison of Porcine S stained membranes with the signal obtained using OTA-HRP showed that only a few of these proteins had an above-average-binding for OTA. This implies certain proteins to have a higher affinity for OTA binding than others. Proteins of approx. 35, 40, 68 kDa, which displayed this characteristic, were detected in all samples tested (male and female). Additional binding-proteins of 20 and 72kDa were detected in rat and human tissue respectively. HREC from both sexes displayed additional proteins of 105 and >250kDa. Similar, but weaker signals were detected with OTB. Addition of 15mM bromophenolblue (BSP), a substance previously shown to inhibit OTA binding, extinguished both the OTA-HRP and OTB-HRP signals. Phenylalanine or aspartame given separately at 15mM or in combination at 15mM, weakened the binding of both conjugates to human kidney proteins and to HRC but not to rat or porcine proteins.

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SPECIES COMPARISON OF RENAL [1H]OCHRATOXIN B PROTEIN BINDING AND UPTAKE IN VITRO.


EUREGIO Ecotoxicology Laboratory, EESL, Konstanz, Germany; Environmental Toxicology, University of Konstanz, Konstanz, Germany; Urology, Klinikum Konstanz, Konstanz, Germany.

The mycotoxic ocheratoxins A and B (OTA, OTB) are known food and feed contaminants produced by certain Aspergillus and Penicillium strains. Chronic dietary intake has been associated with increased incidences of Porcine Mycotoxic Nephropathy, (PMN) and also of uretrothelial tumors (UT) and nephroepithelium in humans (Balkan Endemic Nephropathy, BEN). OTA has also been shown to be carcinogenic in rodents with distinct species and sex-differences. OTB, which differs from OTA only in the substitution of an H-group for a Cl-group, has been shown to be significantly less toxic than OTA both in vivo and in vitro. This fact raises the question whether structure-based differential binding to certain protein structures and/or differential transport mechanisms play a role in ochratoxin toxicity. The aims of this study were to investigate protein binding and uptake of OTB in both sexes of relevant species for comparison with OTA. Such differences could at least in part explain the observed variability in toxicity. [1H]OTA uptake was measured in primary and continuous renal lines from the same species. Similar uptake patterns were apparent as previously noted for [1H]OTA. Protein binding to renal corre-

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COMPARISON OF OCHRATOXIN BINDING TO HUMAN, PORCINE AND RAT RENAL PROTEINS IN VITRO.


Environmental Toxicology, University of Konstanz, Konstanz, Germany; USFDA, Washington, DC; and Urology, Klinikum Konstanz, Konstanz, Germany.

Ochratoxin A and B are found as contaminants in foodstuffs and suspected of being one of the etiological agents responsible for Balkan endemic nephropathy (BEN) and urinary tract tumors. The dechloro-analogue, OTB, is less toxic than OTA in animal studies, suggesting specific structure-activity requirements for toxicity. Ochratoxin A has previously been shown to bind to serum albumin and is also suspected to bind to cellular proteins of the kidney, which could be a prerequisite for toxicity. To detect these renal proteins, OTA and OTB were coupled covalently to horseradish peroxidase (HRP) and were used similarly to an antibody in Western blot analysis. With this method it was investigated if variations in OTA and OTB binding to renal proteins could explain the observed structure-specific and sex-differences. Several proteins from rat, pig and human kidney as well as from proximal human kidney cells (HREC) were detected using OTA-HRP as staining method and displayed large intensity differences. Comparison of Porcine S stained membranes with the signal obtained using OTA-HRP showed that only a few of these proteins had an above-average-binding for OTA. This implies certain proteins to have a higher affinity for OTA binding than others. Proteins of approx. 35, 40, 68 kDa, which displayed this characteristic, were detected in all samples tested (male and female). Additional binding-proteins of 20 and 72kDa were detected in rat and human tissue respectively. HREC from both sexes displayed additional proteins of 105 and >250kDa. Similar, but weaker signals were detected with OTB. Addition of 15mM bromophenolblue (BSP), a substance previously shown to inhibit OTA binding, extinguished both the OTA-HRP and OTB-HRP signals. Phenylalanine or aspartame given separately at 15mM or in combination at 15mM, weakened the binding of both conjugates to human kidney proteins and to HRC but not to rat or porcine proteins.

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DISRUPTION OF GLOMERULAR MESANGIAL AND VISCERAL EPITHELIAL CELL INTERACTIONS IN VITRO BY BENZO(a)PYRENE.


Center for Environmental and Rural Health, Texas A&M University, College Station, TX.

Previous studies in this laboratory have shown that the adaptive response of rat glomerular cells to benzo(a)pyrene (BaP), a prototypic polycyclic aromatic hydrocarbon, is characterized by mesangial cell injury and unregulated proliferation of visceral epithelial cells (i.e. podocytes) in vivo and in vitro. These phenotypic profiles implicate disruption of critical glomerular mesenchymal/epithelial interactions following hydrocarbon challenge. To test this hypothesis, cytochrome (CYP) 1a1 and 1b1 gene expression and cellular proliferation in mesangial/podocyte co-cultures, or in response to conditioned medium, candidate mesenchymal-derived inhibitors of proliferation, including transforming growth factor beta 1 (TGFβ1), prostaglandin E2 (PGE2), and nitric oxide (NO), and matrix cross-plating experiments were conducted in clonal populations of mesangial cells (SCC1) and podocytes (SCC4E) challenged with 3µM BaP. Different inhibitory concentrations of BaP in SCC1 and SCC4E treated with BaP, a pattern consistent with differences in susceptibility to BaP injury. SCC 4E proliferation was inhibited by SCC1 in direct and indirect co-cultures. Denatured conditioned media also inhibited SCC4E proliferation, but no difference between control and BaP groups was observed. TGFβ1 (0.01-0.5 NM), PGE2 (0.02-2 µM) and NO (0.01-1 mM) did not influence SCC4E proliferation. Co-culturing experiments demonstrated that the matrix deposited by SCC1 inhibited SCC4E proliferation. We conclude that disruption of mesenchymal/epithelial interactions by BaP may contribute to the loss of selective permeability induced by BaP in vivo. These alterations are reminiscent of the aberrant proliferation of podocytes observed in several forms of human glomerular disease. (Supported by ES 04917 and ES 09106).
SYSTEMIC UPTAKE OF INHALED ARSENIC IN RABBITS.


Human occupational exposure to sufficiently high levels of arsenic in air has been associated with lung cancer, but generally not other types of cancer. Thus, a better understanding of the relationship between airborne arsenic exposures and systemic uptake is essential. In this study, rabbits were exposed to one of four levels of arsenic trioxide in air for eight weeks (0.05, 0.1, 0.22, or 1.1 mg/m^3). Plasma levels of inorganic arsenic, monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA) were measured following the last exposure. Statistically significant increases in the mean inorganic arsenic levels in plasma were observed only in male rabbits exposed to 0.22 mg/m^3, and in both males and females exposed to 1.1 mg/m^3. Mean inorganic arsenic levels in plasma in males and females exposed to 0.05 and 0.1 mg/m^3, and females exposed to 0.22 mg/m^3, were not significantly elevated compared to controls. These results suggest that arsenic inhalation has a negligible impact on body burden of inorganic arsenic until air levels are significantly elevated. Based on plasma measurements of inorganic arsenic, the two lowest exposure levels in this study (0.05 and 0.1 mg/m^3) are indistinguishable from background. These results have significant implications for risk assessments of inhaled arsenic. To the extent that an exposure has no discernible impact on body burden, attributing an incremental risk or directing risk management actions toward such an exposure would be inappropriate.

INORGANIC ARSENIC BIOTRANSFORMATION AND MITOCHONDRIAL TOXICITY IN HK-2 HUMAN PROXIMAL TUBULAR CELLS.


Arsenic is an environmental toxicant and a human carcinogen. The kidney is a known target organ and is critical for both in vivo arsenic biotransformation and elimination. Thus the toxicity and metabolism of inorganic arsenic was investigated in an immortalized human proximal tubular epithelial cell line, HK-2. Subcellular concentrations of arsenite (<10 µM) and arsenate (<100 µM) were determined by leakage of LDH from cells exposed for 24 hr. The threshold concentration of arsenite (1-10 µM) and arsenate (25-50 µM) on the mitochondria was found by evaluating the metabolism of MTT. Biotransformation of inorganic arsenic was determined in cells exposed to either arsenite or arsenate at the pre-determined subcellular concentrations. Arsenic metabolites were detected in cell culture media and cell lysates using HPLC-ICP-MS, with a limit of detection of 0.5 ppb. No metabolites were detected in lysates or media following 6 hr exposure to either arsenical, indicating that the metabolism of inorganic arsenic in these cells may be a slow event. A 24 hr exposure to arsenite resulted in minimal oxidation to arsenate in media (~5%), and only arsenite was detected in lysates. Exposure to arsenate resulted in its reduction to arsenite (~23%), with both inorganic metabolites detected in the media, and only arsenite detected in lysates. Pentavalent methylated arsénicals were not detected in media following exposure to either inorganic arsenical. However, the arsenic metabolites associated with the protein fraction of cell lysates or media have not been determined, and it is possible that some trivalent methylated metabolites may be present in that fraction. From these results it appears the human renal cell line is capable of biotransforming inorganic arsenic compounds, primarily reducing arsenite to arsenic. In addition, even at low concentrations, the mitochondria are a primary target for toxicity. (NIH ES09490)

DOSE-DEPENDENT BIOTRANSFORMATION OF ARSENITE IN RATS - NOT S-ADENOSYMETHIONINE (SAM) DEPLETION IMPAIRS ARSENIC METHYLATION AT HIGH DOSE.


Arsenic (As) is eliminated by excretion and metabolism. Monomethylarsinic acid (MMAIII) is the most toxic metabolite of arsenic (As), while dimethylarsinic acid (DMAV) produced in the next step is relatively toxic. Methylation of As is carried out by SAM-dependent methyltransferases, which are inhibited by AsIII in vivo. Since the role of methylation in the toxicity and elimination of inorganic As is unclear, we have examined in vivo how excretion and tissue retention of AsIII and its metabolites change with increase of AsIII dose. Rats were injected iv with 20, 50, 125 µmol/kg AsIII and As metabolites in bile, urine and tissues were analyzed by HPLC-hydride generation-flame spectrophotometry. It was found that the excretion of AsIII was about 25% and in urine and tissue concentration increased propor-

MITOCHONDRIA WORK AS REACTORS IN REDUCING ARSENATE TO ARSENITE.


Arsenate (AsV) is a structural analogue of phosphate (Pi), yet its toxic effect is likely due to its reduction to the more toxic arsenite (AsIII). The cellular and molecular mechanisms of this process are still unclear. Since mitochondria take up AsV like Pi, we studied whether these organelles reduced AsV to AsIII, and if they did so, what the characteristics of this process were. Rat liver mitochondria were isolated and incubated with AsV. AsV and its metabolites were then quantified by HPLC-hydride generation-flame spectrophotometry. Analysis of the deproteinized incubates revealed that mitochondria rapidly reduced AsV. Among the substrates supporting the citric acid cycle glutamate enhanced the reduction most effectively. ADP increased, whereas AMP and ATP decreased the AsIII formation. These effects could be prevented by atracyloside, an inhibitor of adenine nucleotide translocator. Electron transport inhibitors (rotenone, antimycin A, azide) and uncouplers (CCCP, dicumarol) abolished AsIII formation. ATP synthase inhibitors (oligomycin, autotin B) strongly but incompletely inhibited AsIII formation. Phosphate decreased AsIII formation in a concentration-dependent manner. Inhibitors of mitochondrial Pi transporters (mercurials, N-ethylmaleimide and butylated hydroxyanisole) were further studied. Compounds transported by the also Pi-moving dicarboxylate carrier (sulfate, sulfite and thiosulfate) caused partial inhibition. AsIII was recovered completely from the supernatant of the mitochondrial incubate, suggesting that mitochondria exported, all of the formed AsIII. Upon subcellularization of mitochondria their AsV reducing activity was practically lost. Summarizing, our findings support the view that mitochondria work as reactors: they take up AsV, rapidly reduce it to AsIII and export this metabolite. Chemical impairment of the mitochondrial function or disruption of structure inhibits the biotransformation of AsV into AsIII in this organelle. (Supported by OTKA and the Hungarian Ministry of Health)

RAT LIVER CYTOSOL REDUCES ARSENATE TO ARSENITE IN THIOL- AND PURINE NUCLEOSIDE-DEPENDENT MANNER.


As liver mitochondria reduce arsenate (AsV) to the more toxic arsenite (AsIII) (see the papers of Schweibert, Nemeth and Gregus), we examined if other cell fractions also carried out this process. Postmitochondrial supernatant (PMSN) was isolated from rat liver and incubated with AsV. AsV and its metabolites were analyzed by HPLC-HG-AFS. We observed that PMSN reduced AsV to AsIII only in the presence of a thiol. Dithiothreitol (DTT) supported the reduction much more effectively than glutathione. Methylation inhibitors, detergents enhanced AsIII formation. PMSN was separated into microsome and cytosol and AsV reducing activities of these fractions were measured. Only the cytosol reduced AsV. Phosphate (Pi) inhibited the cytosolic AsV reducing activity in a concentration-dependent manner. Mercural thiol reagents also inhibited the process, indicating the involvement of a Pi-utilizing SH-enzyme. On searching for a reduction pattern it was found unexpectedly that oxidized pyridine nucleotides (NAD or NADP), but not their reduced forms, increased AsIII formation. Some other purine nucleoside derivatives, but not pyrimidine nucleotides, also increased the rate 2-3 fold. Examination of the effect of nucleosides and nucleobases on AsV reduction yielded dramatic results: purine nucleosides (inosine/methylxantines) increased AsIII formation, whereas pyrimidine bases (hypoxanthine or guanine) decreased it 85-95%. Although the reten-

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guanine) and is inhibited by thiol reagents, the AsV analogue phosphate and purine bases. Identification of the cysteine AsV reductase is presented in the accompanying poster (Gregus and Németh). (Supported by OTKA and the Hungarian Ministry of Health)

407 PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) AS A CYTOSOLIC ARSENATE REDUCTASE.
Z. Gregus and B. Németh. Dept of Pharmacology, University of Pecs, Medical School, Pecs, Hungary.

The findings of the accompanying poster (Németh and Gregus) indicated that the arsenate reductase (ASVR) activity of rat liver cytosol is due to an SH-enzyme that uses phosphate (or its analogue arsenate, AsV) and a purine nucleoside (guanine or inosine) as substrates. Such an enzyme is PNP that catalyses the phosphorolytic cleavage of 6-oxopurines nucleosides according to the following scheme: guanine + glucose 6-phosphate + ATP + arsenate + ADP. Therefore, we tested the hypothesis that PNP is responsible for the thiol- and purine nucleoside-dependent reduction of AsV to AsII by rat liver cytosol. AsII formed from AsV was quantitated with HPLC-hydride-atomic-fluorescence spectrometry analysis of the deproteinized incubates. The following findings support the conclusion that PNP reduces AsV to AsII, using AsV instead of phosphate in the reaction above. Specific PNP inhibitors (1000, BCCX-1777) at a concentration of 1 μM completely inhibited cystosolic AsV activity. During anion-exchange chromatography of cystosolic proteins PNP activity perfectly coeluted with the AsVR activity, suggesting that both activities belong to the same protein.

2. PNP purified from liver microsomes of PNP null mice (the substrate of PNP competing with AsV) purified and guanine and hypoxanthine (products of PNP favoring the reverse reaction), mercapturic acid (substrates of PNP inhibiting PNP) as well as CL-1000 and BCCX-1777 (specific PNP inhibitors). This PNP is responsible for the AsV reductase activity of rat liver cytosol in the presence of DT. Further research shall clarify the mechanism and the in vivo significance of PNP-catalyzed reduction of AsV to AsII.

408 ARSENIC INDUCES ABERRANT GENE EXPRESSION DURING LUNG DEVELOPMENT: APPLICATION TO TOXICOGENOMICS.

J.S. Petrick, 1 F. M. Blachere, 2 O. Selmin, 2 R. B. Runyan 3 and R. C. Lante 3.
1Department of Pharmacology and Toxicology, The University of Arizona, Tucson, AZ, 2Department of Pediatrics, The University of Arizona, Tucson, AZ, 3Department of Nutritional Sciences, The University of Arizona, Tucson, AZ. 4Center for Toxicology, The University of Arizona, Tucson, AZ and 5Department of Cell Biology and Anatomy, The University of Arizona, Tucson, AZ.

Arsenic is a known human carcinogen and exposure has been correlated with an increased incidence of lung cancers. Such studies strongly suggest that the lung is directly targeted by arsenic. Those exposed to arsenic through contaminated food and drinking water include pregnant women, with unknown developmental consequences in the fetal lung. The hypothesis that in utero exposure to inorganic arsenic causes altered gene expression leading to molecular and physiological changes in the lung, Pregnant Sprague-Dawley rats were exposed to 300 ppb sodium arsenite in the drinking water, from embryonic days one to eighteen. Subtractive hybridization of embryonic lung cDNA from control and treated day 18 embryos yielded 248 differentially expressed cDNA clones. Of 154 clones screened, 74 are confirmed differentially expressed by Southern blot hybridization and have been sequenced. Dose and time dependent effects of arsenic on gene expression patterns in developing rat lung will be evaluated using a custom cDNA microarray, including the 248 clones from the subtractive hybridization. This custom microarray is also to include an additional 112 rat cDNA clones. Clones showing differential expression by cDNA microarray will be sequenced and the expression of selected clones will be validated using Northern blotting and PCR. The effects of altered gene expression on lung development will then be assessed using specific probes to show localization of gene and protein expression. (Supported in part by the NIEHS Superfund Basic Research Program Grant P42 ES04940).

409 GENE EXPRESSION PROFILE OF ARSENITE-INDUCED SKIN TOXICITY: MECHANISIC IMPLICATIONS FOR CARCINOGENICITY.

1Harriwnal Microarray Center, NIEHS, Research Triangle Park, NC and 2Laboratory of Toxicology, NIEHS, Research Triangle Park, NC.

Human exposure to arsenic is associated with an increased incidence of cancer, in particular, skin cancer; however, mechanism associated with AsII-mediated toxicity and carcinogenesis at lower levels of exposure remain elusive. Modifications in cell proliferation, oxidative damage, and DNA repair fidelity have been implicated in AsII-mediated toxicity, but the concomitant occurrence of these major events has not been demonstrated in response to arsenic exposure. We hypothesized the involvement of these alterations in arsenic-mediated human toxicity and investigated the time- and dose-dependent effects of arsenic (0.005-5μM, 1-24hr) in normal diploid human epidermal keratinocytes by performing cDNA microarray analysis to elucidate AsII-mediated global gene expression changes. In addition, we examined biochemical (DNA synthesis) and physiological (cellular mitoses) changes in the proliferative response of NIH/3T3 to AsII exposure in order to better correlate alterations in gene expression with cellular function. Our results indicate that AsII exposure decreased transcripts associated with DNA repair (e.g. p53 and MSH5), and increased transcripts indicative of cellular response to oxidative stress (e.g. SOD1 and NAD(P)H oxidoreductase). AsI induced the expression of transcripts indicative of increased programmed cell death (e.g. FAS, TNF, and PKC), oncogenes, and genes associated with cellular transformation (e.g. growth factor). These observations correlated well with DNA synthesis and mitotic measurements as AsI treatment resulted in a dose-dependent increase in cellular mitoses at 24 hr and an increase in DNA synthesis at 48 hrs of exposure. The data presented here shows that arsenic results in gene expression indicative of decreased DNA repair, increased cell proliferation and oxidative stress. Many of the gene expression alterations reported here are indicative of novel pathways perturbed by AsII which we hypothesize to play a major role in its mechanism of carcinogenesis.

410 ACTIVATION OF AP-1 IN UROTSAS CELLS BY METHYLATED TRIVALENT ARSENICALS.

Z. Drobea, 1 L. Japjes, 2 D.L. Thomas 2 and M. Sibbidi 2. 1Pediatrics, UNC, Chapel Hill, NC, 2Center for Environmental Medicine and Lung Biology, UNC, Chapel Hill, NC and NCHEERL, USEPA, Research Triangle Park, NC.

Chronic exposures to inorganic arsenic (iAs) increase risks of various types of cancers in humans. However, mechanisms by which iAs causes cancer are unknown. Methylation is the major pathway for iAs metabolism in humans. Both trivalent and pentavalent As and methylated arsenicals are intermediates or final products in this pathway: iAs → iAsIII → MAAs → MAAsI → DMAAs → DMAAsII. Recent reports have shown trivalent methylated metabolites, methylarsinous acid (MAIII) and dimethylarsinous acid (DMAIII), to be more cytotoxic, genotoxic and more potent enzyme inhibitors than either iAsV or iAsIII, indicating that methylation increases toxic and carcinogenic potency of iAs. To further characterize the role of trivalent methylated metabolites in the toxicity and carcinogenicity of iAs, we have examined the effects of MAAsII, MAAsIII and DMAAsIII on nuclear levels of proto-oncogenes c-jun and c-fos and on the activation of AP-1 in UROtsa cells, an SV-40 immortalized human urothelial cell line. Short-time (0.5 to 2 h) exposures to 0.1 to 5 μM MAAsII, MAAsIII or DMAAsIII significantly increased nuclear levels of p-c-Jun and DNA-binding activity of AP-1. Western blot and EMSA analysis indicated that c-fos was not involved in activation of AP-1 by these arsenicals. Importantly, only MAAsIII and DMAAsIII were considerably stronger inducers of c-jun phosphorylation and AP-1 activation in UROtsa cells than was iAsII. Western blot analyses of the MAPK enzyme family in cells exposed to trivalent arsenicals showed a significant increase in p-ERK but not in p-JNK or p-p38 levels. The increased p-ERK levels was more profound in cells exposed to MAAsIII and DMAAsIII and correlated with increased levels of c-jun. These results indicate that MAAsIII and DMAAsIII, toxic intermediates of arsenic metabolism, activate AP-1 in human urothelial and affect AP-1-dependent gene transcription. (This abstract does not necessarily represent policy of the USEPA.)

411 CARCINOGENICITY OF INORGANIC ARSENIC IN MICE: TUMORS AND PROLIFERATIVE LESIONS OF THE LIVER, ADRENAL, OVARY, UTERUS AND OVODUCT IN MICE EXPOSED TO SODIUM ARSENITE DURING GESTATION.

M. P. Waalkes 1, J. M. Ward 1, and B. A. Dewan 1. 1NCI at NIEHS, Research Triangle Park, NC, 2NCI, Frederick, MD and 3SAIC, Frederick, MD.

Arsenic (As) is a known human carcinogen, but development of rodent models of inorganic As carcinogenesis has been problematic. Since gestation can be a period of high sensitivity to chemical carcinogenesis, we performed a transplacental carcinogenicity study in mice with inorganic As. Groups (n = 10) of pregnant C3H mice received drinking water containing As as sodium arsenite; NaAsO2 at 0 (control), 42.5 and 85 ppm As ad libitum from day 8 to delivery of gestation. These doses were well tolerated. Dams were allowed to give birth, and offspring were weaned at 4 weeks and then randomly put into separate groups (n = 25) of males and females according to maternal dose. Offspring were observed for up to 104 weeks. A complete necropsy was performed on all mice and tissues were microscopically examined. In male offspring, there was a marked, dose related increase in hepatocellular carcino
Differential Sensitivity of Human and Murine Keratinocytes to SodiumArsenite: Effects on Cellular Viability and Proliferation.

K. L. Trouba and D. R. Germolec. NIEHS, Research Triangle Park, NC.

Arsenic is a toxicant and carcinogen that poses a significant health risk in humans. Although the mechanisms contributing to arsenic-induced skin cancer are not completely understood, the general hypothesis is that the arsenic-induced toxicity and mutagenesis are central to the carcinogenicity of this agent. Based on evidence that arsenic has different biological effects on human, mouse, normal, and transformed cells, this study addressed the hypothesis that arsenic-induced toxicity and cell proliferation are differentially modulated in normal human epidermal keratinocytes (NHEK) and immortalized keratinocytes (HaCaT and mouse HEI-HEL). To test this hypothesis, cellular viability was determined by quantifying neutral red uptake, and cell proliferation quantified by measuring [3H]-thymidine incorporation into DNA following short-term sodium arsenite exposure. The effect of glutathione depletion/enhancement on viability following arsenite exposure was also evaluated in addition of l-buthionine (S,S)-sulfoximine (BSO) or N-acetyl cysteine (NAC) to culture media. Results indicate that the Lc20 for arsenite in NHEK, HaCaT, and HEI-30 keratinocytes is approximately 5-10 μM. 3 μM arsenite enhances DNA synthesis >100% in NHEK as compared to control (no arsenite) but has little effect on immortalized keratinocyte DNA synthesis. BSO increases arsenic-mediated cytotoxicity by reducing the Lc20 to approximately 1-3 μM in all cell types examined, whereas NAC attenuates the viability enhancing effects of low concentrations of arsenite in NHEK. These data suggest that endogenous glutathione modulates the negative and positive effects of arsenite on cellular viability. Taken together, these findings indicate that normal human and immortalized human/mouse keratinocytes have differential sensitivities to arsenic-induced DNA synthesis but similar cytotoxicity profiles. These data further suggest that NHEK may provide an accurate in vitro model in which to study the role of oxidative stress in arsenic-induced skin cancer.

Arsenic is an endocrine disruptor, blocking hormone-mediated gene regulation by estrogen and glucocorticoid receptors in vivo.

J. W. Hamilton1, J. E. Bodwell2, R. C. Kaltschmidt1, A. M. Davis1, J. C. Davey3, L. A. Kingsley3, and J. P. Latreille. Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH and 3Physiology, Dartmouth Medical School, Hanover, NH.

Arsenic (As) is an environmental agent of considerable human health concern in the US and around the world. Exposure to elevated drinking water As has been associated with increased risk of several cancers, vascular disease, diabetes, and reproductive/developmental problems. These risks have been shown to be elevated in populations with drinking water arsenic in the range near the current USEPA drinking water standard for the US. We previously reported that As(III) can act as an endocrine disruptor, almost completely blocking hormone-mediated activation of gene expression by the glucocorticoid receptor (GR) in cultured H4IE1I rat hepatoma cells. We investigated these effects further in the current work. At the cellular physiological level, As was shown to block GR hormone-mediated control of H4IE1I cell growth. As was found to bind, preferentially and nearly stoichiometrically to GR at extremely low levels (nM) using [3H]As(III). Using various GR mutants in a GR-deficient H4IE1I-derived cell line, we also observed that the effects of As on GR signaling most closely mapped to the central DNA-binding domain of GR, which is highly conserved among steroid hormone receptors in the nuclear receptor superfamily. Based on these studies, we then examined whether As would disrupt estrogen receptor (ER)-mediated gene regulation. In cultured H4IE1I rat hepatoma cells, 0.1-3 μM As(III) (4-18 h) suppressed estradiol induction of both native and artificial ER binding element (ERE) containing genes. As(III) at non-toxic doses (50-100 μM/l) also strongly suppressed ER-mediated estradiol induction of interleukin-1 gene expression in four day chick embryo liver in vitro. These results indicate that As(III) targets both GR and ER, and possibly other steroid receptors, at doses that are directly relevant to elevated As levels in US drinking water. (NIEHS SBRP ES07373)

Arsenite induces mitotic arrest and apoptosis in SV40-transformed human skin fibroblasts.

J. C. Stevens1, J. L. Reimers Jr., J. G. Pounds1, D. J. Kaplan1, S. C. McNeely1, B. D. Beersse1, P. A. Mathieu1, and M. J. McCabe1. 1Pharmacology & Toxicology, Universities of Louisville, Louisville, KY; 2 Environmental Health Sciences, Wayne State U., Detroit, MI; 3Molecular Biosciences, Pacific Northwest National Laboratory, Richland, WA; and 4Environmental Medicine, U. Rochester, Rochester, NY.

Chronic arsenic ingestion causes skin, bladder, and liver cancer by an unknown mechanism. Arsenite is known to delay transit through M-phase in normal diploid human fibroblasts (NDHF). Compared to cycling or quiescent NDHF, continuously cycling SV40-transformed human fibroblasts (STHF) were observed to be hypersensitive to arsenite toxicity. Based on these observations, the hypothesis that arsenite was investigated. Five 20 μM NaAsO2-induced cytostasis in cycling NDHF but was not obviously toxic to non-cycling NDHF. Higher concentrations were overtly cytotoxic to both cycling and quiescent NDHF. Concentrations as low as 3 μM were seen to be toxic to STHF, PHase microcopy of NaAsO2-treated STHF suggested concentration-dependent accumulation of cells in mitosis and mitotic arrest. Toxicity measured using AlamarBlue indicated the NaAsO2 LD50 was 24.1 μM in cycling NDHF and 3.5-4.3 μM in the two STHF lines tested. Arsenite treatment of STHF with 0-10 μM NaAsO2 induced concentration-dependent inhibition of cell proliferation, accumulation of cells with G2/M DNA content, and increased mitotic index. Induction of apoptosis was indicated by membrane blebbing observed by phase microscopy, annexin V binding and by electron microscopy showing highly vacuolated cells containing chromosomes surrounded by intact blebbed cellular membranes. These results indicate that SV40 transformation sensitizes cells to arsenite induced mitotic arrest and induction of apoptosis in the mitotic cells. Supported by NIH grants R25 RR12242, P30E050635, R01 ES05460, R01 ES056892 and Commonwealth of Kentucky Research Challenge Trust.

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416 TOXICITY OF PENTAVALENT AND TRIVALENT ARSENICALS IN HUMAN UMGBLICAL VEIN ENDOTHELIAL CELLS: ROLE OF PH.
W. A. Port, S. A. Benjamin and R. S. Yang. Center for Environmental Toxicology & Technology, Colorado State University, Fort Collins, CO.

Chronic exposure to low levels of arsenic (As) in the environment has been associated with disorders of vascular endothelial cells (ECs) and a variety of cancers, including hepatic angiosarcoma, in which the cell of origin is the hepatic sinusoidal EC. The mechanism underlying As-induced EC dysfunction and its carcinogenic effects remain unexplored. Under both physiological and pathological conditions, ECs can be exposed to an acidic microenvironment, which is known to influence the progression of many diseases, including cancer. To investigate whether an acidic microenvironment mediates the toxic effects of As on ECs, we evaluated, under conditions of normal physiological pH and acidsis, the toxicities of trivalent and pentavalent inorganic As and pentavalent methylated As metabolites. Human umbilical vein endothelial cells (HUVECs) were exposed to arsenite (As(III)), arsenate (As(V)), dimethylnitrosamine ([DMAs(V)]), or dimethylarsinic acid ([DMAs(V)] at concentrations in the milli- and micromolar ranges for 24 hours under conditions of 5% CO(2) or 20% CO(2), to achieve a culture medium pH of 7.4 or 7.0, respectively. HUVEC viability was assessed using the MIT assay. Preliminary results indicate that at pH 7.4, As(III), with an LC(50) of 30 μM, was more toxic to HUVECs than As(V), MAs(V), or DMAs(V), with LC(50) of 7.25, and 50 mM, respectively. At pH 7.0, the LC(50) of As(III), MAs(V), and DMAs(V) were similar to those at pH 7.4 (18, 15, and 75 mM, respectively). However, at pH 7.0, the LC(50) of As(III) was 125 μM, approximately four times higher than at pH 7.4. These results suggest that acidsis may modulate the toxicity of trivalent As species in HUVECs. Evaluation of the toxicity of pentavalent methylated As metabolites is also planned. This project is funded by the NCI (K08 CA72596-05) and ATSDR (U61/AT11881-05).

417 POTENTIAL CELLULAR MECHANISMS FOR ARSENIC-INDUCED VASCULAR DISEASES.
N. V. Sougy, A. Barchewski, L. R. Kli, J. W. Hamilton and M. A. Ihnert.

Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH and Cell Biology, University of Oklahoma Medical School, Oklahoma City, OK.

Chronic, low level exposure to arsenic increases the incidence of proliferative vascular diseases, such as arteriosclerosis and ischemic heart disease. Arsenic-associated changes in blood vessels may contribute to the vascular components of diabetes and tumor growth. Angiogenesis is fundamental to many of these pathological changes and may underlie the vascular actions of arsenic. In support of this hypothesis, arsenite (0.033-1.0 μM) significantly increased blood vessel growth in an in vitro chicken embryo model. Above 1.0 μM, arsenite inhibited blood vessel growth. To examine potential mechanisms for these vascular effects, expression of the angiogenic genes, vascular endothelial cell growth factor (VEGF) and plasminogen activator inhibitor-1 (PAI-1), was examined in porcine aortic smooth muscle cell (SMC) cultures. Concentrations of up to 100 μM arsenite caused SMC to proliferate. Release of VEGF protein increased following a 24 h exposure to 5-25 μM arsenite. By 48 h, 1 μM arsenite caused significant VEGF release. Dose and time-dependent changes in VEGF and PAI-1 mRNA levels were consistent with the changes in protein levels. However, VEGF expression continued to increase above 50 μM arsenite, while PAI-1 mRNA levels peaked at 2.5 μM arsenite and dropped to control levels by 10 μM. Arsenite levels induced the hypoxia inducible factor (HIF) protein levels in a manner consistent with enhancement of the VEGF and PAI-1 promoter activity. Inhibitor studies demonstrated that arsenite stimulated oxidant formation and kinase cascades to increase HIF levels and angiogenic gene expression. Microarray analysis in human airway epithelial cells, demonstrated that exposure to arsenite (5 or 50 μM) for 4 h increased HIF mRNA levels by 1.8-3 fold. These data suggest that arsenite causes specific, dose-dependent effects on cell signaling that promote angiogenic responses, which contributes to pathologic vascular changes. Supported by ES07373.

418 EVALUATION OF IN VIVO ACUTE IMMUNOTOXICITY OF AN INORGANIC ARSENICAL, SODIUM ARSENITE, USING MURINE MODELS.
T. Sakurai and K. Fujisawa. School of Life Science, Tokyo University of Pharmacy and Life Science, Tokyo, Japan. Sponser: M. Walsh.

It has been well known that inorganic arsenicals are potent toxicants and carcinogens in humans and most animals. However, immunotoxic effects of them have not been well studied. In this study, we observed in vivo acute immunotoxicity of an inorganic arsenical, arsenite, using murine models. Sodium arsenite was administrated intraperitoneally to male CDF1 mice aged 4 weeks to doses of 1.25-8.0 mg/kg (body weight) on day 0 and multiple immune functions of these mice were assessed until day 16. As results, a half numbers of mice were died when 8 mg/kg of arsenite were administered. The mice body weights were decreased significantly by arsenite in a dose dependent manner at doses of 1.25-4.0 mg/kg on days 1-2, and it returned to the control level on day 4. Administered arsenite was distributed to various organs, including spleen, thymus, liver, lung, kidney and heart, with similar kinetics determined by AAS; the maximum arsenite concentrations were detected on day 1 and they dropped slowly to the control level during 16 days. The weights of immune organs, spleen, thymus and liver, were significantly decreased by arsenite at doses of 1.25-4 mg/kg on days 1-2, and they returned to the control levels on day 4. However, it did not affect on lung, kidney, heart and brain weights. 4-mg/kg arsenite also decreased significantly the numbers of splenocytes, thymocytes, peritoneal exsudate cells, peripheral blood leukocytes and erythrocytes of mice on temporary day 1, but it did not affect on alveolar macrophage and Peyer’s patch lymphocyte numbers. Cellular lysosomal enzyme activity and nitric oxide production of peritoneal macrophages, and bladegoness of splenocytes and thymocytes were inhibited by 4-mg/kg arsenite on day 1 although the functions of alveolar macrophages, Peyer’s patch lymphocytes and bone marrow cells were not changed. Taken together, arsenite has significant in vivo immunotoxicity both quantitatively and qualitatively, and the immune organs are very sensitive to arsenite inhalations.

419 ARSENIC-INDUCED SELF-TOLERANCE AFTER CHRONIC EXPOSURE IN HUMAN PROSTATE EPITHELIAL CELLS.

NCI at NIHES, Research Triangle Park, NC and Michigan State University, East Lansing, MI.

While acquired self-tolerance to arsenic (As) has been shown in many rodent cell lines, acquisition of As resistance in human cells appears difficult to induce. Acquired As tolerance is linked to a decrease in glutathione(GSH) levels, particularly by increase in glutathione S-transferase (GST) activity and the participation of efflux pumps such as MRP and MDR. Thus, we studied chronic exposure of a human prostate cell line (RWPE-1) and development of As tolerance. RWPE-1 cells were continuously exposed to 0 or 5 μM arsenite (As(III)) for 18 or more weeks. Acute toxicity of As(III) (600 μM; 48 h) was then assessed using the MTS assay. The acute LC(50) of As(III) in chronic As-exposed prostate epithelial (CASE-PE) cells was 43.8 μM whereas 17.6 μM in control cells, a 2.5-fold increase. CASE-PE cells grown in As-free medium for 4 weeks regained As-resistance, indicating a stable change in phenotype. As accumulation over 24 h in cells exposed to 10 μM As(III) was markedly reduced in CASE-PE cells (5.4 μg As/10(6) cells) compared to control cells (65.6 μg As/10(6) cells). When As-loaded cells were placed in As-free media for 2 h, the rate of As efflux was 2.5-fold greater in CASE-PE cells than control. CASE-PE cells showed increased GSH levels (4.9-fold) and increased GST activity (1.4-fold). GSH depletion with buthionine sulfoximine or inhibition of GST with ethacrynic acid both abolished As tolerance in CASE-PE cells. Although As-tolerant cells did not show changes in MRP or MDR gene expression, the transcription levels of P-glycoprotein and ABCG2, and the expression of P-glycoprotein, P-glycoprotein, and ABCG2 genes in MRP and MDR, respectively) both abolished As tolerance. Thus, human prostate epithelial cells chronically exposed to As acquire tolerance to As. The mechanism of this tolerance appears to involve increases in GSH levels and GST activity, but not increases in MRP or MDR gene expression, although the functionality of these pumps is key to maintaining resistance.

420 IS THERE A SPECIAL SENSITIVITY OF CHILDREN TO INORGANIC ARSENIC?

Gradent Corporation, Waukeene, WI, Gradent Corporation, Mercer Island, WA and Gradent Corporation, Cambridge, MA.

For some chemical exposures, the period of early childhood represents a stage of development characterized by increased sensitivity. There has been recent interest in the possibility that enhanced child susceptibility to the toxic effects of arsenic. This work evaluated the available evidence addressing this concern. While metabolic differences between children and adults have been noted, the evidence is contradictory. For example, while a study in Northern Argentina observed some differences between children and adults, a study of children in Washington State did not observe age-related differences in arsenic metabolism. Studies of the health effects of arsenic in children suggest that these differences may provide a more relevant basis for discerning age-related differences in susceptibility. In a Chilean population of children exposed to high levels of arsenical, dermal effects (i.e., hyperkeratosis, leuko-melanoderma) were associated with an estimated daily arsenic intake of 0.063 mg/kg-day. This dose-effect level lies within the dose range derived from studies reporting similar effects in adults, i.e., 0.01 to 0.1 mg/kg-day. Other findings of specific health effects in children, including hearing changes and neurological deficits, are inconsistent. These studies also have limitations which preclude determining a definitive role for
421 THE PROTECTIVE EFFECTS OF ASCORBIC ACID AND FOLIC ACID ON ACUTE ARSENIC TOXICITY IN CULTURED MOUSE FIBROBLASTS.

L. Z. Crandall, R. H. Finnen and R. V. Vose. Pharmacology University of Nebraska Medical Center, Omaha, NE and Institute of Biotechnology and Genomics, The Texas A&M University System Health Science Center, Houston, TX.

The acute cytotoxic effects of arsenic can be modulated by a number of factors, including folic acid supplementation, glutathione status, and prior exposure to this metalloid. Based on the hypothesis that protective factors reverse arsenic-induced oxidative stress and oxidant detoxifications in cellular membrane metabolism, this study was designed to determine if the nutritional factors ascorbic acid (AA) and folic acid (FA) protect cultured cells against arsenic toxicity. Using the neutral red cytotoxicity assay, it was found that the antioxidant ascorbic acid at 10 and 30 μM significantly decreases the toxicity of 3 and 6 μM sodium arsenite (As) in C3H10T1/2 fibroblasts. To determine if arsenic toxicity is influenced by in the folic acid transporter Folbp2, primary fibroblast cell lines were created from Folbp2−/−, +/+ and −/+ embryos; the genotype of each cell type was corroborated by PCR screening. When exposed to As for 24 hours, Folbp2 null fibroblasts exhibit slightly more cytotoxicity than do wild type or heterozygous fibroblasts. The addition of 30 μM FA to Folbp2 null cells did not afford protection against arsenic toxicity. In contrast, the toxicity of sub-lethal concentrations of As was attenuated by FA in wild type cells. These results corroborate our earlier finding that FA protects SWV/Fb fibroblasts from acute arsenic toxicity. In addition, it appears that this protective effect is dependent upon a functional Folbp2 gene. Because Folbp2 null mice exhibit an increased susceptibility to arsenic-induced neural tube defects, it is proposed that compromised Folbp2 function is a risk factor for arsenic-dependent neuronal toxicity secondary to compromised uptake of FA. Because exposure through drinking water and food is a major problem in many countries whose populations commonly deficient in FA and AA, it is proposed that supplementation of these two nutrients might protect against As toxicity.

422 HEPATOPROTECTIVE EFFECTS OF SEMISULCOCHLORA LIBERTINA AND GARLIC ON THE LIVER DAMAGE INDUCED BY CARBON TETRACHLORIDE IN RATS.

T. W. Jeon, E. S. Lee, K. J. Kim and H. J. Kim. Efficacy and Safety Research Center for Traditional Oriental Medicines, Kyung University, Daegu, South Korea and College of Oriental Medicine, Kyung University, Daegu, South Korea.

Semisulcospira libertina (Korean freshwater snail) has been used as a folk medicine for the treatment of various diseases such as jaundice, stomachache, dyspepsia, and diabetes in the Eastern Asia. Also, garlic has many medicinal properties. This study was designed to examine hepatoprotective effects of S. libertina and garlic on the acute hepatotoxicity of carbon tetrachloride (CCL4). Male Sprague-Dawley rats weighing 200-220 g were pretreated with dehydrated powder of S. libertina (2.1 g/kg, po SI) and dehydrated powder mixture of S. libertina and garlic (3 g/kg, 73 ratio, po SQ) once daily for 3 consecutive days, and then a single toxic dose of CCL4 (1 g/kg, 5 μl/kg) was administered at 24 hr later. Liver damage was assessed by quantitating serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH) and alkaline phosphatase (ALP) activities as well as histopathological examination. Pretreatments with SI and SG significantly decreased CCL4-elicited ALT (48 %, 61 % respectively), AST (32 %, 67 %), and SDH (51 %, 76 %) activities, but had no effect on ALP. SL and SG have revealed hepatoprotective effects against CCL4-induced histopathological changes such as severe necrosis, inflammatory cell infiltration and congestion in the central zone of hepatic lobule. SG enhanced the hepatic content of glutathione at 106 % compared with CCL4 group, but SI increased to a somewhat lesser extent (65 %). These findings demonstrate that SL and SG have the hepatoprotective effect on CCL4-induced liver damage.

423 HEPATOPROTECTIVE EFFECTS OF SEMISULCOCHLORA LIBERTINA AND GARLIC ON ACETAMINOPHEN-INDUCED LIVER DAMAGE IN MICE.

T. W. Jeon, E. S. Lee, Y. S. Lee, O. K. Han, K. J. Kim and H. J. Kim. Efficacy and Safety Research Center for Traditional Oriental Medicines, Kyung University, Daegu, South Korea and College of Oriental Medicine, Kyung University, Daegu, South Korea.

Semisulcospira libertina (Korean freshwater snail) has been used as a traditional drug for the therapy of various diseases such as jaundice, stomachache, dyspepsia, and diabetes in the Eastern Asia. Since the legendary medicinal properties, garlic has attracted scientific interests for several decades. The purpose of this study was to investigate the hepatoprotective effects of S. libertina and garlic on liver damage induced by acetaminophen (AA). Male ICR mice weighing 30-32 g were pretreated with dehydrated powder of S. libertina (3.5 g/kg, po SI) and dehydrated powder mixture of S. libertina and garlic (5 g/kg, 7.5 ratio, po SQ) once daily for three consecutive days, given a single toxic dose of AA (450 mg/kg, in saline, ip) and liver function determined 18 hr later. Liver damage was assessed by quantitating serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and sorbitol dehydrogenase (SDH) activities as well as by histopathological examination. Pretreatments with SI and SG dramatically lowered AA-elicited ALT (94 %, 97 %, respectively), AST (88 %, 89 %), and SDH (93 %, 93 %) activities. SI and SG have revealed hepatoprotective effects against AA-induced histopathological changes. SI enhanced the hepatic content of glutathione by 43% compared with control animals. The gene expression of TNFα mRNA on AA-induced liver damage was determined by RT-PCR. SL and SG markedly decreased the gene expression of TNFα mRNA induced by AA in mice. These findings demonstrate that SI and SG have the hepatoprotective effect on AA-induced liver damage.

424 EXTRACT OF ASTRAGALI RADIX DECREASES ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN MICE.

Y. S. Lee, O. K. Han, T. W. Jeon, E. S. Lee, K. J. Kim and H. J. Kim. Efficacy and Safety Research Center for Traditional Oriental Medicines, Kyung University, Daegu, South Korea.

Astragalus radix (AR) is one of the oldest and most frequently used crude drug for traditional medicine in many Asian countries. This study investigated the hepatoprotective effects of the hot-water-extracted AR against acetaminophen (AA)-induced hepatic damage in ICR mice. AA at the dose of 450 mg/kg ip produced liver damage in ICR mice. Pretreatments with AR significantly decreased serum enzyme activities of alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase. However, concentration of hepatic glutathione did not show a significant change between the tested groups. We also investigated TNFα mRNA gene expression on AA-induced liver damage by RT-PCR. AA drastically induced TNFα mRNA gene expression in ICR mice. Pretreatment of mice with AR led to a marked decrease of TNFα mRNA gene expression. These data indicate that the protective effect of AR against AA-induced hepatotoxicity in mice appears to be related to the regulation of TNFα mRNA expression as well as biochemical alterations.

425 OLEANOLIC ACID INCREASES INOS AND TNF-α GENE EXPRESSION IN MACROPHAGES BY NF-KB ACTIVATION.

C. Y. Choi, J. Y. Kim, S. J. Park, K. J. Lee and H. G. Im. Pharmacy and Research Center for Proteins Materials, Chosun University, Kwangju, South Korea, and Biologics Chesterman National University, Kwangju, South Korea.

Oleanolic acid (OA), a pentacyclic triterpenoid acid, is reported to have anti-tumor activities; however, the mechanism underlying its anti-tumor effects is poorly understood. To further determine the mechanism of OA, we investigated the effects of OA on the release of NO and TNF-α, and on the level of iNOS and TNF-α gene expression in mouse macrophages. We found that OA elicited a dose-dependent increase in NO and TNF-α Production. Reverse transcription polymerase chain reaction showed that the increased NO and TNF-α secretion were due to an increase in iNOS mRNA and TNF-α mRNA, respectively. Since iNOS and TNF-α transcription has recently been shown to be under the control of the NF-kB transcription factor, the effects of OA on NF-kB activation were examined using a transient transfection assay and an electrophoretic mobility shift assay (EMSA). Transient expression assays with NF-kB binding sites linked to the luciferase gene revealed that the increased level of iNOS mRNA and TNF-α mRNA induced by OA were mediated by the NF-kB transcription factor complex. Using DNA fragments containing the NF-kB binding sequence, OA was shown to activate the protein/DNA binding of NF-kB to its cognate sites as assessed by EMSA. These results demonstrate that OA stimulates NO and TNF-α release and is able to upregulate iNOS and TNF-α expression through NF-kB transactivation, which may be the mechanism whereby OA elicits its biological effects.

426 ALPHA-HEDERIN AUGMENTS NITRIC OXIDE PRODUCTION AND GENE EXPRESSION IN MURINE MACROPHAGES.

H. G. Jeong. Pharmacy and Research Center for Proteins Materials, Chosun University, Kwangju, South Korea.

Alpha-Hederin (HD), a triterpene saponin, is reported to have anti-tumor activities; however, the mechanism underlying its therapeutic effects is not known. In this study, we examine the effects of HD on the release of nitric oxide (NO) and the
level of inducible nitric oxide synthase (iNOS) gene expression in mouse macrophages. HD elicited a dose-dependent increase in NO secretion. Reverse-transcription polymerase chain reaction showed that the increased NO secretion was due to an increase in iNOS mRNA. Transparent expression assays with NF-kB binding sites linked to the luciferase gene revealed that the increased level of iNOS mRNA induced by HD was mediated by the NF-kB transcription factor complex. These results demonstrate that HD stimulates NO release and is able to upregulate iNOS expression through NF-kB transactivation, which may be a mechanism whereby HD elicits its biological effects.

427 PREVENTION OF ACETAMINOPHEN-INDUCED LIVER TOXICITY BY Platycodon grandiflorum IN MICE.

K. J. Lee1, S. J. Park2, Y. S. Kim1, Y. C. Chung1, J. K. Seo1 and H. G. Jeong1, 1Biolog, Chonnam National University, Kangju, South Korea, 2Pharmacy and Research Center for Medicinal Material, Chosun University, Kangju, South Korea, 1Korea Research Institute of Chemical Technology, Taejon, South Korea, 3Food Nutrition, College of Chinv, Chinv, South Korea and 4R&D, Jangseung Dongai Co., Ltd., Chinv, South Korea.

The protective effects of an aqueous extract from the roots of Platycodon grandiflorum A. DC (Campanulaceae), Changhil (CK), on acetaminophen (APAP)-induced hepatotoxicities and the possible protective mechanisms involved were investigated in mice. Pretreatment with CK prior to the administration of APAP significantly prevented the increase in serum alanine aminotransferase and aspartate aminotransferase activity and hepatic lipid peroxidation in a dose-dependent manner. APAP-induced hepatotoxicity was also essentially prevented as evidenced by liver histopathology. Hepatic glutathione levels and glutathione-S-transferase activities were not affected by treatment with CK alone; however, pretreatment with CK protected the APAP-induced depletion of hepatic glutathione levels. The effects of CK on cytochrome P450 (P450) 1A2 and 2E1, the major isoenzymes involved in APAP bioactivation, were investigated. In micromolar incubations, CK effectively inhibited P450 1A2-dependent methoxyresorufin O-deethylase activities and the P450 2E1-dependent p-nitrophenol and aniline hydroxylase. The results suggest that the protective effects of CK against the APAP-induced hepatotoxicity may, at least in part, be due to its ability to block P450-mediated APAP bioactivation. (This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea: HMP-00-B-21600-0117)

428 THE HERBAL PRODUCT Schizandra chinensis PROTECTS AGAINST LIPOPOLYSACCHARIDE (LPS)-INDUCED HEPATOTOXICITY IN FISCHER 344 RATS.


Estimates are that sales of herbal medicines exceeded $4 billion in 1999. A number of herbs are said to have beneficial effects on the liver but scientific evidence of their safety, efficacy and mechanism of action is limited or lacking. Schizandra chinensis (Schizandra) has traditionally been used in China for various ailments, from cough to anemia, and has been touted as a treatment for liver disease. We previously reported the anti-inflammatory effects of Schizandra. After 24h, blood for preparation of serum was collected via heart puncture and one lobe of the liver was harvested and fixed in 10% buffered formalin. The other lobe was precision-cut and slices incubated at 37°C for 72h in a Viron incubator. At 24, 48 and 72h, liver slices were harvested, weighed and assayed for potassium (K) and lactate dehydrogenase content as indices of viability. Treatment with Schizandra enhanced maintenance of viability (K content) in liver slices from rats exposed to LPS compared to slices from rats treated with LPS alone. Potassium levels in rats treated with Schizandra and LPS were increased 31% (50 μl dose), 35% (200 μl dose) and 20% (500 μl dose) in liver slices harvested at 48h. There were no significant differences in the weights of the rats in the different groups. These findings demonstrate that Schizandra administered in vivo, under the conditions tested, protected liver slices against LPS-induced hepatotoxicity. (Supported by NIAMS Center Grant P085506-694 (JU), the Richard B. Siegel Foundation (JU, PN) and the UA Undergraduate Biology Program (CK, ZL, DC, NYT).)

429 INHIBITION OF 2-AMINO-1-METHYL-6-PHENylimidazo[4,5-b]PYRIDINE-ASSOCIATED COLORECTAL CARCINOSIS BY NATURAL FOOD ANTHOCYANINS IN RATS INITIATED WITH 1, 2-DIMETHYLMORPHAZINE.

A. Hagiwara1, H. Yoshihara2, M. Sato3, S. Tanamo1, T. Koda1, M. Nakamura1, N. Itoh1 and T. Shirai1, 1Daioi-Ai Institute of Medical Science, Ishinomiya, Japan, 2First Department of Pathology, Nagoya City University, Nagoya, Japan, 3Satsu-En Gijutsu 1F Inc., Toyota, Japan and 4Nagoya City University, Nagoya, Japan.

The potential of natural anthocyanin food colors, purple currant color (PCC), purple sweet potato color (PSPC) and red cabbage color (RCC), to modify colorectal carcinogenesis was investigated in male F344/DuCJ rats, initially treated with 1, 2-dimethylhydrazine (DMH) and receiving 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the diet. After DMH initiation, PCC, PSBC and RCC were given at a dietary level of 5.0% in combination with 0.02% PhIP until week 36. No anthocyanins-treatment-related changes in clinical signs and body weight were found. Incidences and multiplicity of colorectal adenomas and carcinomas in rats initiated with DMH (25%; 0.3) were clearly increased by PhIP (93%; 2.7). In contrast, lesion development was pronouncedly suppressed by PCC administration (30%; 0.3), and also moderately inhibited by PSBC (70%; 1.4) or RCC (50%; 1.0) treatment. Furthermore, in the non-DMH initiation groups, induction of aberrant crypt foci (ACF) by PhIP was significantly decreased by the RCC supplementation. The results thus demonstrate that while PhIP clearly exerts promoting effects on DMH-initiated colorectal carcinogenesis, these can be reduced by PCC, PSBC or RCC in diet, under the present experimental conditions.

430 EFFECT OF DOSE, TIME, AND ROUTE OF ADMINISTRATION OF GENISTEIN ON RADIATION PROTECTION.

M. R. Lander1, V. Srinivasan1, A. Shapiro1, C. H. Takimoto2, P. S. Wang3 and T. M. Seed1, 1Armed Forces Radiobiology Research Institute, Bethesda, MD and 2National Cancer Institute, Bethesda, MD.

Radioprotective agents have applications in clinical and military settings, space flight, and radiation site cleanup. The estrogenic and antioxidative properties of the soy isoflavone genistein (Gen) suggest that it is an ideal radiation protective candidate. This research investigated the radioprotective efficacy of genistein in CD12F1 male mice. Exp. 1: Mice were each administered a single subcutaneous (sc) dose of genistein either 1 or 24h before a lethal dose of gamma radiation (9.5 Gy cobalt-60 at 0.6 GY/min). In both groups, mice either received saline, polyethylene glycol 400 (PEG) vehicle, or genistein at 3, 6, 12, 25, 50, 100, 200, or 400mg/kg body weight. The 30-day survival rates in the groups treated 1h before irradiation were 8%, 13%, 13%, 9%, 0%, 6%, 6%, 6%, 13%, and 19%, respectively. Survival rates in the groups treated 24h before irradiation were 8%, 15%, 6%, 0%, 19%, 60%, 56%, 56%, 95%, and 85%, respectively. Thus, genistein did not provide radioprotection at any of the doses when given 1h before irradiation, doses of 25-400 mg/kg given 24h before 9.5 Gy afforded significant protection. Exp. 2: Mice that each received a single oral gavage of saline, PEG, or 400 mg/kg genistein 1h before 9.5 Gy irradiation had 30-day survival rates of 0%, 9%, and 3%, respectively; when given 24h before 9.5 Gy, survival rates were 3%, 9%, and 9%, respectively. Thus, a single oral dose of genistein was not radioprotective. Exp. 3: Mice received daily gavages of 100- or 400-mg/kg genistein for either 4 days before, 4 days after, or 4 days before and after irradiation at 9.5 Gy. Survival rates of mice receiving 100-mg/kg genistein were 0%, 0%, and 50%, respectively; for those receiving 400- mg/kg genistein, survival rates were 44%, 0%, and 69%, respectively; and for those receiving a saline and PEG, survival rates were 0% and 19%, respectively. This research demonstrates that both a single sc dose and multiple oral doses of genistein protect mice from a lethal dose of gamma radiation.

431 SILYMARIN ATTENUATES THE HEPATOTOXICITY OF ALPHAP-NAPHTHYLISOCOTYLANE.

D. A. Hill, S. Edwards, T. V. Martin, B. Gotham and Y. Hiji, Biology, Morgan State University, Baltimore, MD.

Silymarin, a polyphenolic flavonoid derived from milk thistle seeds, demonstrates anti-inflammatory and hepato-protective activity against certain toxicants. In rodents, the acute hepatotoxicity of alpha-naphthylisocotylane (ANIT) is inflammatory cell-dependent and mimics that of drug-induced cholestasis in humans. Treatment with silymarin has anti-inflammatory activity and recent studies suggest that it decreases the formation of ANIT-induced hepatic lesions, the hypothesis that silymarin affords protection against ANIT-induced hepatotoxicity was investigated. Male Sprague-Dawley rats were treated for 3 consecutive days with 0.5-0 mg/kg silymarin (oral). On day 6, the silymarin treated rats received 120 mg/kg ANIT (oral). Parameters of hepatotoxicity were determined 12 and 24 hours after ANIT.

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432 DEVELOPMENTAL EXPOSURE OF RATS TO DIETS CONTAINING PACIFIC OCEAN OR LAKE OHIO SALMON MODIFIES LATER RESPONSES TO LPS.

N. M. Filipov1, K. O. Brench, Y. Xu1, M. C. Craig-Schmidt1, D. A. Lawrence2 and R. E. Segal1.

1Wadsworth Center, NY State Department of Health, Albany, NY.
2CCEH, Basic Sci., College of Vet. Med., Miss. State University, Mississippi State, MS.

Fish contain fatty acids (FA), some of which (i.e. n-3) may be beneficial. However, there are large variations in FA content and spectrum between fish species as well as in the same species from different sources. Because n-3 FA are anti-inflammatory, we sought to determine whether developmental exposure to diets containing salmon or salmon-extracted oil from either Pacific Ocean (PO) or Lake Ontario (LO) would, in the adult, modify the inflammatory response to LPS. From gestational day 6 through weaning, dams were fed diets containing 20% lypophilized PO or LO salmon, PO or LO oil derived from equivalent amounts of salmon, or control chow. Females offspring, 4 months old, were given LPS (100 μg/kg BW, i.p.) and blood was collected for up to 5 h. In control rats, LPS increased serum tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6), and nitric oxide (NO). However, in offspring of dams fed fish oil from either PO or LO, the TNFα increase after LPS was diminished by 40%. At 3 h post LPS, rats fed PO oil diet had serum IL-6 lower than the rest. At 5 h, although greater than controls, serum IL-6 in rats fed PO oil was lower than in rats exposed to LO oil. Serum NO at 5 h post LPS was reduced by 70% only in the rats fed PO oil diets. Effects of PO and LO lypophilized salmon diets on serum cytokines and NO after LPS were similar, but less pronounced. PO analysis revealed that PO oil has 5-fold higher n-3/n-6 FA ratio than LO oil. In summary, PO oil is a more potent anti-inflammatory agent than LO oil due, at least in part, to the higher n-3 FA content of PO oil; these effects are long-lasting and are partially masked by other dietary constituents of fish. Thus, dietary FA manipulation early in life may alter sensitivity to inflammatory stimuli in adulthood.

(Supported by NIEHS ES079102 and EPA 82381201 to RFS; NIEHS ES05879 to NMP).

433 HUMAN PROSTATE CANCER CELLS TREATED WITH SAW PALMETTO EXTRACTS SELECTIVELY ACCUMULATE A BROWN PIGMENTED MATERIAL: CORRELATION WITH ALTERED CELLULAR PROLIFERATION AND MORPHOLOGY.


MB Research Laboratories, Spinnerstown, PA.

To determine the direct effect of commercially available saw palmetto berry extract on the viability and proliferation of human prostate cancer cells in culture, cultures of the human prostate cancer cell line, LNCaP, were treated with increasing concentrations of aqueous extracts of saw palmetto oil. Cultures of a transformed human epithelial keratinocyte cell line (HaCaT) were treated in a similar manner for comparison. Following a 24-hour treatment period, the cells were labeled with bromodeoxyuridine and the viability and proliferation of the treated cells were determined using standard flow cytometric methods. Treatment of LNCaP cells with aqueous extracts of saw palmetto oil resulted in a concentration and time-dependent accumulation of a brown pigment within the cytoplasm of the cells. The appearance of this material within the cells was associated with alterations in cellular morphology and a dramatic decrease in the percentage of proliferating cells, without a major effect on the viability of these cells. The development of pigmented material and its association with the inhibition of proliferation was much more pronounced in the HaCaT cell line, occurring at 10-fold higher concentrations. The results of the current study indicate that the beneficial effects of saw palmetto on pathophysiological condition of the prostate may be associated with the selective accumulation of this pigment material in treated prostate cells and its inhibitory effect on cellular proliferation.

434 EFFECTS OF COMBINED QUERCETIN AND COENZYME Q10 ON THE OXIDATIVE STRESS PATHWAY IN NORMAL AND STREPTOCOCCUS-INDUCED DIABETIC RATS.


Medical Sciences, Indiana University School of Medicine, Bloomington, IN.

Reactive oxygen species may be involved in the genesis of a number of pathological states such as ischemia-reperfusion injury, cancer, and diabetes, as well as in aging. The objective was to determine if combined dosing of quercetin and coenzyme Q10 (10mg/kg/day ip for 14 days) is beneficial or detrimental in the oxidative stress pathway of 30 day streptococcus-induced diabetic Sprague-Dawley rats. Blood glucose concentrations were elevated in both normal and diabetic rats after quercetin and coenzyme Q10 treatment. Liver tissue were examined for the presence of oxidized species of glutathione: GSSG and GSSP, glutathione remnants, with and without treatment. No significant difference was noted in the oxidized species of glutathione. Liver tissue were examined for the presence of oxidized species of glutathione: GSSG and GSSP, glutathione remnants, with and without treatment. No significant difference was noted in the oxidized species of glutathione. In kidney, tissue were examined for the presence of oxidized species of glutathione: GSSG and GSSP, glutathione remnants, with and without treatment. No significant difference was noted in the oxidized species of glutathione. In kidney, tissue were examined for the presence of oxidized species of glutathione: GSSG and GSSP, glutathione remnants, with and without treatment. No significant difference was noted in the oxidized species of glutathione. In kidney, tissue were examined for the presence of oxidized species of glutathione: GSSG and GSSP, glutathione remnants, with and without treatment. No significant difference was noted in the oxidized species of glutathione.

435 OXIDATIVE STRESS PARTICIPATES IN ACUTE LUNG INJURY AND ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES (MAPK) FOLLOWING AIR POLLUTION PARTICLE EXPOSURE (PM).

F. S. Roberts1, J. Jasko2, J. Richards3 and K. J. Derbez1.

1College of Veterinary Medicine, NC State University, Raleigh, NC and 2NIEHS, USEPA, Research Triangle Park, NC.

Epidemiological reports suggest that PM is associated with increased rates of cardiopulmonary-related morbidity and mortality. In vitro studies have demonstrated insights into the mechanism of lung injury caused by PM. To further understand the mechanism of PM injury, we studied the in vivo effects of residual oil fly ash (ROFA) exposure on MAPK activation. The MAPK cascade regulates the activity of specific nuclear transcription factors that control genes involved in cell growth, differentiation, apoptosis, and inflammation. A 2x2 factorial arrangement of treatments was used to randomize design (n=26 male Sprague-Dawley rats, 60-90 d of age). Factors included: 1) pre-treatment with either an intraperitoneal (IP) injection of 500 mg/kg dimethylthiourea (DMTU) or saline, thirty minutes prior to instillation (IT) and 2) IT with 0.5 mg/rat ROFA or saline. Bronchoalveolar lavage fluid was obtained at 24h for determination of cytotoxicity and inflammation. ROFA increased lactate dehydrogenase and induced neutrophilia at 24h post-exposure (P < 0.05), IP pre-treatment with DMTU inhibited both the cytotoxicity and neutrophilia (P < 0.05). Thirty-six male Sprague-Dawley rats (60-90 d of age) were then randomly assigned as previously described and whole lung samples were collected for analysis at 1h, 3h, and 24h. ROFA increased p-ERK levels at 3h and 24h (P < 0.05). These increases were attenuated by DMTU pre-treatment (P < 0.01). DMTU inhibited the ROFA induced p-ERK activity increase at 24h (P < 0.01). The inhibition of ROFA-induced injury, MAPK expression and MAPK activity by DMTU provides strong evidence that generation of oxidative stress is critical to ROFA-induced cytotoxicity. These data link in vitro and in vivo results and demonstrate an oxidative mechanism operating through molecular signaling pathways by which environmental factors cause lung injury. (This abstract does not reflect EPA policy).

436 SUB-CHRONIC EFFECT OF METAL WORKING FLUIDS ON PULMONARY FUNCTIONS IN B6C3F1 MICE: ROLE OF VITAMIN E.


PBB, NIOSH, CDC, Madison, WI.

Metal working fluids (MWFs) are widely used in many industries. Exposures to MWFs are known to cause a spectrum of respiratory effects like excessive cough and attendant secretory products, bronchitis and physical discomfort as evidenced
by tightness of chest. This leads us to examine if an inhalation exposure to a semisynthetic nebulizer MWF produces any morphological as well as functional alterations in the lungs of mice. Morphological-functional and antioxidant outcomes in lungs were studied after inhalation exposure to MWF of vitamin E-deficient and sufficient mice. Mice were given vitamin E deficient or basal diets for 35 weeks. Inhalation exposure to MWF started after 18 weeks on respective diets. Mice were exposed to MWF for 85 individual exposures (17 weeks, five days a week, 6 hours a day). No morphological alterations were seen in the lungs of mice from the studied groups. However, functional deficits in breathing rate, peak inspiratory/expiratory flow, minute ventilation, and tidal volume, along with marked reduction in the lung levels of GSH, protein thiol and acetoacetate were in evidence in mice maintained on vitamin E-deficient diet. Mice maintained on sufficient diet and exposed to MWF had decreased tidal volume compared to respective controls. Exposure to MWF resulted in accumulation of peroxidative products, and reduction of acetoacetate content in the lungs compared to vitamin E sufficient or deficient controls. The results of this study indicate that dietary vitamin E deficiency enhanced subchronic effect of inhalation exposure to MWF.

437 MECHANISMS OF UV INDUCED FREE RADICAL OXIDATIVE DAMAGE IN HUMAN SKIN FIBROBLASTS.
J. L. Matta 1, M. Morales 1, R. A. Armstrong 1 and H. D’Antoni 1.
1 Pharmacology/Toxicology, Ponce School of Medicine, Ponce, Puerto Rico; 2 University of Puerto Rico, Mayaguez, Puerto Rico and 3 AMES Research Laboratory, NASA, Moffett, CA. Sponsor: J. Matta.

UV radiation induces the production of oxygen free radicals although there is limited understanding of mechanisms by which UV induces oxidative damage in human skin fibroblasts. We tested the hypothesis that acute exposure to environmental levels of UV radiation in Ponce School of Medicine isogenics cause oxidative damages to fibroblasts leading to apoptosis and necrosis. Environmental UV levels were measured over a period of two and half years and used to establish irradiation doses for human fibroblasts (CRL-1072) using an Oriel 1 KW UV Solar Simulator. Cells were irradiated with doses of 0.28 (low dose), 0.36 (medium dose) and 0.44 (high dose=5.4 min) [J/cm2] and DNA adducts, DNA repair capacity, lipid peroxidation, apoptosis and necrosis measured. A low dose did not cause changes in guanosine and 2-deoxyguanosine (2DG). However, the mean dose resulted in increases of 40% and 43% in guanosine and 2DG respectively. The high dose caused an increase of 35.1% and 34% in guanosine and 2DG, respectively and a 245% increase in lipid peroxidation. The DNA repair capacity of these cells increased after acute exposure to low and mean doses but decreased with a high UVA/UVB dose. Acute exposure to the equivalent of 1.5 hours of environmental levels of UV caused necrosis in all fibroblast cell lines. These results show that even a short-term, acute exposure to environmental levels of UV can cause substantial oxidative damage resulting in apoptosis and necrosis. Supported by NASA grant NAG-2-1176 to J. L. M.

438 N-ACETYLCysteine (NAC) PRETREATMENT DECREASES COCAINE AND ENDOTOXIN INDUCED HEPATOTOXICITY.
R. A. Lalez, R. M. Turkel and M. S. Abdel-Rahman. Pharmacology & Physiology, University of Medicine and Dentistry of New Jersey, Graduate School of Biomedical Sciences, Newark, NJ.

Cocaine (COC) produces hepatotoxicity by a mechanism, which remains undefined, but has been linked to its oxidative metabolites. Endotoxin (lipopolysaccharide, LPS) is also a well-known cause of hepatic damage, due to glutathione (GSH) depletion and oxidative stress. Previously we have demonstrated that exposure to non-injurious doses of LPS dramatically increases cocaine mediated hepatotoxicity (CMH). N-Acetylcysteine (NAC) protects against oxidative stress and depletion of GSH. This study was conducted to investigate if NAC pretreatment inhibits LPS potentiation of CMH. For five consecutive days, male CF-1 mice were pretreated with oral NAC (400 mg/kg) or saline, followed an hour later with COC (20 mg/kg) or sterile saline. On the last day, four hours after the last COC or saline treatment, the mice were administered 12 x 104 EU LPS/kg or sterile saline. The mice were sacrificed 18 hours later by decapitation. For the COC alone and COC + LPS groups, NAC pretreatment, significantly decreased serum ALT and AST activities, indicating reduced liver injury. In addition, the blood GSH content in the COC alone and COC + LPS groups pretreated with NAC was 25% higher versus the mice pretreated with saline. Moreover, in all groups pretreated with NAC, hepatic GSH concentration significantly increased as well as the hepatic and blood glutathione peroxidase (Gpx) and catalase (Cat) activities. In conclusion, the results demonstrate that NAC pretreatment exerted a protective effect against LPS potentiation of CMH.

439 AMELIORATION BY PHYSICAL CONDITIONING OF CARDIAC OXIDATIVE INJURY CAUSED BY CHRONIC NITRIC OXIDE SYNTHASE INHIBITION IN RAT.
K. Husain, T. M. Boyce and S. R. Hazzelrig, Surgery, Southern Illinois University School of Medicine, Springfield, IL.

Many individuals with cardiac disease undergo periodic physical conditioning with or without medication. Therefore, this study investigated the interaction of physical training and chronic nitric oxide synthase (NOS) inhibitor (Nitro-L-Arginine Methyl Ester, L-NAME) treatment on blood pressure (BP), heart rate (HR) and cardiac oxidative/antioxidant in rats. Fisher 344 rats were divided into four groups and treated as follows: 1) sedentary control, 2) Exercise training for 8 weeks, 3) L-NAME (10 mg/kg, s.c. for 8 weeks) and 4) ET + L-NAME. The animals were sacrificed 24 hr after last treatments and hearts were isolated and analyzed. Physical conditioning resulted in a significant increase in respiratory exchange ratio (RER), cardiac NO production, NOS activity and endothelial NO synthase (eNOS) and inducible iNOS protein expression. Training significantly enhanced cardiac GSH levels, GSH/GSSG ratio and up-regulation of cardiac CuZn-SOD, Mn-SOD, catalase (CAT) glutathione peroxidase (GSH-Px) activity and protein expression. Training also caused depletion of cardiac malondialdehyde (MDA) level, protein carbonyl content. Chronic L-NAME administration resulted in depletion of cardiac NO level, NOS activity, eNOS, nNOS and iNOS protein expression, GSH level, GSH/GSSG ratio and down-regulation of cardiac CuZn-SOD, Mn-SOD, CAT, GSH-Px activity and protein expression. Cardiac nitrite oxide (NO) activity increased with enhanced MDA level and protein oxidation after L-NAME administration. The biochemical changes were accompanied by increased in BP and HR. Interaction of training and chronic NOS inhibitor treatment resulted in normalization of BP, HR and up-regulation of cardiac antioxidant defense system. It is concluded that physical conditioning could reduce injury caused by chronic NOS inhibition by up-regulating the cardiac antioxidant defense system and lowering the BP and HR in rats supported by AHA/IA grant # 00513952.

440 α-TOCOPHEROL OR INDOMETACIN PRETREATMENT PREVENTS SEIZURE-INDUCED INCREASES IN F2 ISOPROSTANES AND CITRULLINE.
D. Mihalovets, R. C. Gupta 1, W. D. Dentburn 1, T. Vajlly-Nagy 1, J. Morrow 2 and T. Montine 1.
1 Pathology, Vanderbilt University, Nashville, TN; 2 Pharmacology, Vanderbilt University, Nashville, TN. Medical, Vanderbilt University, Nashville, TN and Murray State University, Hopkinsville, KY.

Free radicals, particularly the reactive oxygen or nitrogen intermediates, are thought to be involved in kainic acid (KA) toxicity, exacerbating excitotoxicity and exerting tissue damage. F2-isoprostanes (isoP) and F2-isocitrulline (isoC) are highly accurate biomarkers of oxidative injury and are products of free radical-catalyzed peroxidation of arachidonic acid and docosahexaenoic acid, respectively. Activation of nitric oxide synthase (NOS), resulting in an increase of nitric oxide (NO) and citrulline, has also been suggested to be part of KA toxicity. As a measure of oxidative stress, lipid peroxidation and NOS activity changes in isoP and citrulline levels were studied in the brain cortex and striatum of rats pretreated with α-tocopherol (100 mg/kg, i.p.) for 3 days or those exposed to indometacin (inhibitor of cyclooxygenase, COX, 14 μg/ml of drinking water) for 2 weeks prior to KA treatment. These findings provide novel information about KA-induced excitotoxicity and the relationship of seizures to free radicals, COX and NO. (Supported by AG 05144, AG 16835 and ES05497).

441 IMMUNOCHEMICAL DETECTION AND LOCALIZATION OF MALONDIALDEHYDE-PROTEIN ADDUCTS IN THE SPIELEN S OF ANILINE-TREATED RATS.
X. Wu, D. L. Boor and M. F. Khan, Pathology, University of Texas Medical Branch, Galveston, TX.

We have reported previously that aniline exposure in rats causes increased lipid peroxidation and formation of malondialdehyde (MDA)-protein adducts in the spleen. In order to further elucidate the role of MDA-protein adducts in the splenic toxicity of aniline, studies were conducted to detect and localize these adducts in the spleen. Rabbit polyclonal antisera to MDA-KLH were employed for Western blot analyses and immunohistochemical localization of MDA-protein adducts in the spleens of aniline-treated (65 mg/kg/day aniline in the drinking water for 30 days) and control rats. Western blot analysis of splenic microsomal proteins from aniline-treated and control rats showed the presence of 13 MDA-modified proteins. However, 26, 32 and 44 kDa proteins were more prominent in the aniline-

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treated rats. For immunohistochemical localization of MDA-protein adducts in the spleen, a new approach using alkaline phosphatase-fast red (red color) demonstration of bound primary antibodies was adopted, providing a sharper and increased contrast compared to horseradish peroxidase-diaminobenzidine (brown color) methodology. This new approach allowed us to differentiate the changes in aniline-treated spleens, which had extensive brownish deposits of iron. Spleens from aniline-treated rats showed intense staining for these adducts in the red pulp areas (where iron was also localized), especially within the sinusoidal macrophages. Spleens from control rats showed only mild staining. The co-localization of MDA-protein adducts with iron in the red pulp of the spleen provides a potential mechanism of splenic toxicity of aniline.

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**BENZO(A)PYRENE-QUINONES (BPQs) GENERATE REACTIVE OXYGEN SPECIES (ROS) AND ARE MITOGENIC IN LYMPHOMA AND NON-LYMPHOMA CELLS.**

A. D. Burdiek1, K. J. Liu1, M. K. Walker1, M. D. Reed1, M. Morske1, F. T. Laufer1 and S. W. Burdiek1, 1College of Pharmacy, Toxicology Program, The University of New Mexico, Albuquerque, NM and 2Lovelace Respiratory Research Institute, Albuquerque, NM.

Previous studies have shown that quinones (diones) of benzo(a)pyrene (BaP) are formed in the presence of ultraviolet light and under the influence of various P450s and peroxidases. We have shown that BPQs are mitogenic in human lung epithelial cells (Reed et al, submitted) and human mammary epithelial cells (Burdiek et al, submitted). We have also shown that BPQs generate reactive oxygen species (ROS) as detected using fluorescent ROS-reactive substrates including DCHF and DHE.

In this presentation, we will compare the effects of BPQs on lung and mammary epithelial cells. In addition, we present new data indicating that BPQs are highly mitogenic in murine spleen cells, including those obtained from Ahr-/- null mice. In these studies, Ahr-/- spleen cells treated with 0.1-1 μM 3, 6-BPQ, 1, 6-BPQ, and 1, 12-BPQ had nearly a tenfold increase in cell proliferation in a 3-day T cell mitogenesis assay. BaP (0.1-1 μM) was not immunosuppressive to T cell mitogenesis in Ahr-/- spleen cells. The results of these studies demonstrate that mild oxidative stress in various lymphoid and nonlymphoid cells is mitogenic, perhaps owing to activation of the epidermal growth factor receptor (EGFR) in epithelial cells or other mitogenic receptors in lymphoid cells. This work was supported in part by NIH grants: ES-05495, ES-07293, ES-10433, and P20 ES-0987.

**444**

**ACRYLONITRILE POTENTIATES NOISE-INDUCED HEARING LOSS: A CONSEQUENCE OF OXIDATIVE STRESS.**

D. B. Rao, Z. Debebe, S. Kist and L. D. Fechter, Center for Toxicology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Previous research from this laboratory (Rao, 2000; 2001) has shown that exposure of oxidative stress by asphyxiation + noise results in cochlear injury and auditory impairment. In this study, we sought to determine whether acrylonitrile, an agent that significantly depletes glutathione would also promote noise-induced hearing loss. Acrylonitrile is commonly used in the production of plastics and synthetic fibers. It also ranks among the top 50 high volume chemicals produced in the US alone. Although acrylonitrile is a suspected human carcinogen, the neurotoxic effects are not clearly understood. In this study, we determined the effects of acrylonitrile on auditory function and also on cochlear glutathione levels. Long Evans hooded rats were exposed to acrylonitrile, noise alone, and acrylonitrile + noise, and no experimental treatment (controls). Following a recovery period of three weeks, auditory thresholds were tested electrophysiologically. Results demonstrate that the auditory thresholds from animals administered acrylonitrile and exposed to noise had much higher thresholds than animals exposed to noise alone. Acrylonitrile produced no adverse effects on auditory function by itself. Spectrophotometric assay of glutathione levels in the cochleas following acrylonitrile administration demonstrates a marked reduction of glutathione compared to controls. We hypothesize that acrylonitrile potentiates noise-induced hearing loss by rendering the cochlea more sensitive to oxidative stress. Supported in part by ES08092.

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**OXIDATIVE STRESS INDUCED IN MURINE SPLENOCYTES AFTER EXPOSURE TO MULTIPLE PESTICIDES IN VITRO.**

H. P. Mills1, C. L. Rabideau1, F. Madhavan2 and H. Chandra2, 1Biomedical Sciences and Pathology, Virginia Tech, Blacksburg, VA and 2Office of Research and Development, USEPA, Cincinnati, OH.

The widespread use of pesticides in the environment increases the likelihood of multiple pesticide exposure, either concurrently or sequentially, over an organism's lifetime. This study examines both pre-oxidant and antioxidant status in murine (C57BL/6) splenocytes exposed to individual pesticides and pesticide mixtures, in vitro. The three insecticides of interest are fenthion (Lyn, sin octachloro, malathion (Mal, an organophosphate), and malathion (Mal, an organophosphate), and piperonyl butoxide (PBO, a synergist). These three pesticides were found to be cytotoxic to splenocytes in both concentration- and time-dependent manner. Based on minimum cytotoxicity (≤ LC50), the following concentrations had been chosen for this study: 70μM M, 50μM Mal, and 50μM PBO. The pre-oxidant and antioxidant status of the cells were monitored as indices of oxidative stress. The pre-oxidant hydrogen peroxide (H2O2) activity was measured using the dichlorofluorescin diacetate assay. Exposure to pesticides for 15 minutes significantly increased H2O2 production above the controls: Mal 211.1%, Lind 18.8%. PBO 25.9%. Mal-Lind 26.8%. Mal + PBO 37.8%. The activities of superoxide dismutase and catalase levels in splenocytes were not significantly altered by these pesticides and their mixtures even when exposed for 12 hrs. The use of glutathione reductase (GSH-Red) and glutathione peroxidase (GSH-Px) levels were significantly reduced when exposed to pesticide mixtures. Thus, GSH-Rx levels were found to be control 51.7, Mal 48.2, Lind 50, PBO 52.3, Mal + Lind 40.5, Mal + PBO 42 Units/mg. The GSH-Px activity levels were found to be control 44.9, Mal 30.2, Lind 30.6, PBO 32.4, Mal + Lind 21.1, Mal + PBO 21.1 Units/mg. These results indicate that exposure to mixtures of Mal, Lind and PBO...
446 THE OXIDATIVE STRESS LEVELS OF WORKERS WHO HAVE POSSIBLE EXPOSURE TO MUNICIPAL INCINERATOR FLY ASH.


It is well known that municipal solid waste incinerator (MSWI) fly ash includes heavy metals, dioxins, polycyclic aromatic hydrocarbons (PAH), and other organic materials. According to in vitro and animal studies, heavy metals and dioxins probably cause oxidative stress and PAH may also cause oxidative stress during metabolism in human body. In this study, we measured the levels of oxidative stress markers in the blood and urine specimens of MSWs workers and discussed whether the duration of exposure to MSWI fly ash was associated with the levels of oxidative stress markers. The subjects were 81 male workers (mean age 42.7) from four MSWIs in the same city. Written informed consents were given from all subjects. Occupational health doctors interviewed each subject about his job history, evaluated the exposure to fly ash, and calculated its total duration. The subjects were classified into four groups; those were Long duration of exposure to fly ash, Short duration of exposure to fly ash, Limited exposure to fly ash, and Control. Blood and urine specimens were obtained from subjects in the morning. The levels of 8-hydroxydeoxyguanosine (8-OHdG) in urine and in leukocytes were measured as oxidative DNA damage makers. Blood malondialdehyde (MDA) and lipid peroxides (LPO) levels, and total urinary biopyruvates level were also measured as oxidative stress markers. α-Tocopherol was measured as major antioxidant in serum. The mean levels of all markers were compared among four groups. Although the levels of all markers had no significant differences among groups, the tendency that the level of urinary 8-OHdG elevated with the increased duration of exposure to MSWI fly ash was significant. Considering this result, some chemicals consisted in MSWI fly ash might have induced oxidative stress in the workers.

447 MODULATION OF PMN FUNCTION BY INCUBATION WITH HUMAN PLASMA FROM OXIDANT-CHALLENGED INDIVIDUALS.

E. Hofer1, A. Tabak, Y. Baum2, A. Tamir2, Y. Lerman1 and T. Machamid2. 1Israel Poison Information Center, Rambam Medical Center, Haifa, Israel. 2Dept Epidemiology, Faculty of Medicine Technion, Haifa, Israel. 3Occupational Health, Sacker Faculty of Medicine, Tel Aviv, Israel 4Occupational Medicine, Kupat Holim Sick Fund, Ashdod, Israel.

Polymorphonuclear leukocytes (PMN) play a fundamental role in the primary host defense against bacterial pathogens. It is possible that PMN function might be modulated by soluble immune mediators released into the blood stream due to oxidative stress. PMN from healthy volunteers were tested for the stimulated release of superoxide anions after being incubated with plasma of: 1. Welders and a matched (age and smoking habits) non-welders group 2. Smokers and non-smokers 3. Elderly and young individuals. The experiments showed that stimulated superoxide production was inhibited (p<0.05) in plasma from smokers as compared to that of non-smokers and by plasma from elderly individuals as compared to that of younger ones. There was no statistical difference between superoxide release from PMN incubated with plasma from welders as compared with that from an age and smoking habits non-welders matched group. These findings indicate that age and smoking induce the appearance in plasma of factors which affect neutrophil function. Determination of these factors could elucidate the mechanism of impaired immune response in elderly and oxidant-challenged individuals.

448 INDUCTION OF METALLOTHIONEIN SYNTHESIS BY MITOCHONDRIAL OXIDATIVE STRESS.

M. Kondo, N. Putakawa, M. Takiguchi, M. Higashimoto and M. Sato. Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Mitochondria is a major source of reactive oxygen species (ROS). The harmful effects of mitochondrial ROS have been implicated in the aging process and neurodegenerative diseases, but antioxidant systems against mitochondrial ROS are not fully understood. Metallothionein (MT), a cysteine-rich and metal-binding protein, is an efficient scavenger of oxygen radicals, such as superoxide and hydroxyl radicals, but the induction of MT by mitochondrial ROS have never been investigated. In this study, we examined the induction of MT synthesis by administration of mitochondrial-specific ROS generators such as antimycin A (AA), an electron transfer inhibitor, and 2, 4-dinitrophenol (DNP), an uncoupling agent. Subcutaneous administration of AA or DNP to mice significantly increased the hepatic MT concentration in a close- and time-dependent manner. Administration of AA or DNP increased hepatic lipid peroxide production, and AA elevated mitochondrial lipid peroxide production in the liver. DNP or AA treatment elevated the hepatic lipid peroxidation and serum glutamine-photore transaminase (GPT) levels. The inhibition of gluthathione (GSH) synthesis by L-buthionine-SR-sulfoximine (BSO) enhanced the liver injury caused by DNP or AA administration. Moreover hepatic MT concentrations of DNP- or AA-treated mice were increased by BSO-pretreatment. The intracellular accumulation of hydroperoxide by AA or DNP treatment in MT-/- fibroblasts cells was higher than that in MT+/+ cells, suggesting that MT plays a role in regulation of intracellular reactive oxygen levels caused by mitochondrial stress. Taken together, these results suggest that mitochondrial oxidative stress induces MT synthesis, which may contribute to regulation of production of mitochondrial reactive oxygen.

449 GENERATION OF REACTIVE OXYGEN SPECIES IN BREAST-CANCER CELL LINES AFTER TREATMENT WITH CLARIFIED SLURRY OIL AND CRUDE OILS.

B. Yilmaz1, D. O. Carpenter1, J. Srempekwa1, C. R. Mackerer2 and K. E. Arencot1. 1School of Public Health, University at Albany, Rensselaer, NY. 2Environmental Science, University of Massachusetts, Amherst, MA and 3CSC, Consulting in Toxicology, Princeton, NJ.

Clariified slurry oil (CSO), and two crude oil samples, Belridge Heavy Crude Oil (BHCO) and Lost Hills Light Crude Oil (LHLCO) were examined for their ability to generate reactive oxygen species (ROS) in human breast-cancer (MCF-7) cell cultures. Oil samples were prepared in DMSO resulting in extraction of virtually all of the aromatic hydrocarbons including the 3-7 ring polyaromatic compounds (PACs) comprising 62.2% of the CSO, 9.0% of the BHCO, and 2.0% of the LHLCO by total weight. Generation of ROS was measured in a Coulter Epic flow cytometer using 20 µM dihydrothreoxide 123 (DHR) and cell viability was determined using 5 µM propidium iodide (PI). In the acute treatment experiments, single-cell suspensions were loaded with DHR and PI and then treated with the oil samples (1 ppm or 10 ppm). Measurements were made at 5, 15, 30, 60 and 90 minutes after addition of oil samples. In the chronic experiments, pre-confluent cell cultures were treated with oil samples for 6, 12, or 24 hours prior to preparing single-cell suspensions that were loaded with DHR and PI. Acute treatment with CSO, BHCO or LHLCO resulted in no increase in the generation of ROS as compared to the positive control, hydrogen peroxide. Chronic treatment with the CSO sample resulted in a significant increase in ROS at 24 hours. Treatment of cell cultures with a single polyaromatic hydrocarbon, BENZO(A)PYRENE, resulted in a significant and concentration-dependent generation of ROS at 24 hours. (Supported by NIEHS Award RO1 ES049795-01A1 and TUBITAK-NATO)

450 OXIDATIVE DAMAGE TO RAT ERYTHROCYTE MEMBRANE LIPIDS AND SKELETAL PROTEINS BY MAQ-NOH, AN N-HYDROXY METABOLITE OF THE ANTIMALARIAL DRUG, PRIMAQUINE.

L. J. Bolchoz, D. C. McMillan and D. J. Jollow. Dept. of Pharmacology, Medical University of South Carolina, Charleston, SC.

We have shown previously that 6-methoxy-8-hydroxyxanomolinoine (MAQ-NOH), a potential human metabolite of the antimalarial drug, primaquine, is a direct-acting hemoglobinolytic and hemolytic agent in rats. To examine the mechanism underlying the hemolytic activity of MAQ-NOH, the present studies were undertaken to determine the effect of MAQ-NOH on rat erythrocyte sulfhydryl status, membrane lipids and skeletal proteins, and morphology under conditions known to commit the cells to premature removal from the circulation. Treatment of rat erythrocytes with MAQ-NOH (350-750 µM) depleted GSH with concomitant formation of glutathione-protein mixed disulfides. MAQ-NOH also induced a concentration-dependent formation of thiobarbituric acid-reactive substances and F2-isoprostanes, indicating the occurrence of lipid peroxidation. SDS-PAGE and image analysis of erythrocyte gels using an anti-hemoglobin antibody revealed that MAQ-NOH treatment had no effect on membrane skeletal proteins, despite a concentration-dependent increase in the content of membrane-bound hemoglobin monomer. Furthermore, scanning electron microscopy revealed that MAQ-NOH had no effect on red cell morphology. The data are consistent with the concept that MAQ-NOH induces erythrocyte membrane damage by an oxidative stress, however, the mechanism underlying its hemolytic activity may differ from that of other hemolytic N-hydroxylamines, such as dapsone hydroxylamine. Supported by NIH grant AI46424.
and protected against Fe(III)-induced MITO permeability transition. In addition, hepatocytes isolated from TS pretreated rats were resistant to toxic oxidative damage caused by MITO toxins such as rotenone and tetrahydrofuranato, mitochondrial complex I and II inhibitors respectively, in combination with the glutathione inhibitor, diethyl maleate. In contrast, acute T administration, at an equimolar concentration enriched liver homogenates but not MITO or IIMM with T and did not protect against MITO toxin-induced oxidative hepatocyte death, indicating that mitochondria are a critical site for oxidative damage leading to acute cell death. We suggest that TS acts as a unique delivery system for T, rapidly accumulating in MITO and IIMM to prevent mitochondrial damage and cell death. We propose that the acute administration of TS may prove useful for the therapeutic treatment or prevention of oxidative stress-mediated acute liver injury. Supported by NIH grant #ROI ES05452 and Gaster and Irene Lazzara Foundation

454 CYCLOOXYGENASE-2 CONTRIBUTES TO OXIDATIVE STRESS INDUCED BY TEMPORARY FOCAL BRAIN ISCHEMIA.

Focal cerebral ischemia is associated with a local inflammatory response that participates in the progression of ischemic brain injury. Cyclooxygenase-2 (COX-2) exacerbates ischemic injury of the brain. COX-2 catalyzes the formation of prostaglandins from arachidonic acid (AA) via cyclooxygenase/peroxidase cycle. Peroxidase reactions can generate radicals independent of AA metabolism. Thus COX-2 may contribute to oxidative stress during temporary focal brain ischemia. We found that total antioxidant reserves of rat brain as well as concentrations of ascorbate and GSH were decreased after 6 and 24 h of temporary focal brain ischemia in rats. A selective COX-2 inhibitor, SC58125, prevented depletion of ascorbate and GSH in ischemic rat brain. Next, we used COX-2 null and wild type mice to investigate the role of COX-2 in the accumulation of cerebral injury. We found that MCA occlusion induced a significant decrease of ascorbate and GSH in COX (+/+) but not in COX-2 (-/-) mice. In COX (+/+) mice, ischemia produced higher steady-state concentrations of ascorbate radicals in brain homogenates than in COX (-/-) mice or controls. Addition of AA to brain homogenates resulted in additional increase of ascorbate radical EPR signal. In line with this, the ascorbate radical EPR signal was significantly lower in brain homogenates of rats pretreated with SC58125 before ischemia (SC58125 did not affect ascorbate radical in the presence of a non-specific redox catalyst, Fe2+). Overall, our data suggest that COX-2 contributes to the generation of free radicals and oxidative stress after temporary focal brain ischemia.

455 PEROXYNITRITE FORMATION PRECEDES CELL DEATH IN MIDZONAL NECROSIS DURING LOW FLOW HYPOXIA TO PERFUSED RAT LIVERS.
J. R. Blattner, R. T. Carrin', R. Schoonhoven' and J. J. Lemasters'.
1: Curriculum in Toxicology, UNC-CH, Chapel Hill, NC; 2: Developmental and Cell Biology, UNC-CH, Chapel Hill, NC; 3: Environmental Sciences and Engineering, UNC-CH, Chapel Hill, NC.

Low flow perfusion of isolated rat livers results in early hypoxic hepatocellular necrosis at the border between normoxic periporal and anoxic pericentral hepatocytes. This midzonal region is postulated to have increased superoxide production. Nitric oxide and superoxide undergo a diffusion-limited reaction to form peroxynitrite, a potent oxidant that has been shown to be produced in ischemic heart immediately after reperfusion. Our aim was to assess peroxynitrite formation in perfused rat livers during low flow hypoxia. Rat livers were perfused via the portal vein with Krebs-Heinslert bicarbonate (KHB) buffer saturated with 95% O2/5% CO2 at 3.4 ml/min of flow. After 20 min of normoxic perfusion, flow rates were decreased to 0.5 ml/min for varying times (30, 60 and 120 min). Subsequently, the livers were infused with KHB containing 0.5 mM cyanide blue for 10 min and then with 2% paraformaldehyde in KHB for fixation. To detect peroxynitrite generation, nitrotyrosine adducts were identified by immunohistochemistry using rabbit polyclonal anti-nitrotyrosine antibody and a peroxidase detection system. After 30 min of hypoxia, an increase in hepatocellular nitrotyrosine labeling was observed in midzonal regions. Nitrotyrosine labeling increased further at one hour and was observed in both viable and non-viable hepatocytes, as assessed by trypan blue uptake. After two hours, nitrotyrosine labeling and necrosis expanded from midzonal regions to pericentral regions. Nitrotyrosine labeling occurring in both viable and non-viable hepatocytes, we conclude that peroxynitrite formation began prior to cell death. We also conclude that both nitric oxide and superoxide are generated at the border between anoxic and normoxic tissue, which leads to peroxynitrite formation. This peroxynitrite generation may contribute to early midzonal necrosis during low flow hypoxia in the liver.
D456 DELAYED INTERACTIVE EFFECTS OF SARIN, PYRIDOSTIGMINE AND EXERCISE ON THE HEPATIC ANTIOXIDANT DEFENSE SYSTEM IN MICE.


Pyridostigmine (PYR), a reversible cholinesterase inhibitor, has been employed as a pretreatment drug for possible nerve agent exposure during the Gulf War. The Gulf War veterans were exposed to risk factors such as pesticides, medical agents and possible low levels of nerve gas. Physical activity increases hepatic ROS production, leading to oxidative stress and damage. It is hypothesized that under stressful conditions during war, PYR and/or sarin could affect the antioxidant system in liver. Hence, this study investigated the interactive effects of PYR, low-dose sarin and exercise training on hepatic antioxidant defense system in mice. Male NIH Swiss mice were divided into eight groups and treated as follows: (1) Control; (2) Sarin [0.01 mg/kg, 1/10 LD50]; (3) Exercise training; (4) Sarin plus exercise; (5) PYR [1.2 mg/kg, p.o.]; (6) PYR plus exercise; (7) PYR plus Sarin; (8) PYR plus Sarin plus exercise. Exercise was given for 10 weeks, however PYR and sarin were administered daily during 5th and 6th weeks. The animals were sacrificed 24 hours after the last treatments and livers isolated. SOD activity significantly decreased in groups 3 and 6 compared to control. Catalase/Carotene activity were reduced significantly to 70% and 71% of control in groups 5, 6, and 8 respectively. GSH/PH activity significantly reduced (70% of control) in Group 6. GR activity decreased in Groups 3 and 6 compared to control. Malondialdehyde significantly increased 124% and 173% of control in Groups 3 and 5 respectively. This study suggests that exercise (physical activity) plus PYR lead to significant alteration in liver antioxidants and lipid peroxidation, which may produce oxidative injury in hepatic tissue of mice. (In part supported by US Army contract DMDA 17-97-C-70 and SIU School of Medicine)

D457 FORMATION OF OXYGEN RADICALS AND RELEASE OF NITRIC OXIDE (NO) IN RATS UPON EXPOSURE TO HIGH LEVELS OF BLAST OVERPRESSURE: IS NO A MODERATOR OF CIRCULATORY OXIDATIVE STRESS?

N. V. Cortunov. Respiratory Research, Walter Reed Army Institute of Research, Silver Spring, MD.

Exposure to blast overpressure (BOP) has been previously shown to result in lung injury characterized by alveolar rupture and pulmonary hemorrhage leading to oxygen insufficiency and oxidative stress (OxS) to the circulatory and cardiovascular systems. These events may include a compensatory release of endogenous nitric oxide (NO), a vasodilator and antioxidant that, in turn, would decrease the extent of OxS via reduct interaction between NO and circulatory oxygen radicals (OXR).

To further examine this hypothesis, deeply anesthetized Sprague-Dawley rats were exposed to BOP shock waves of magnitude 100-10 kPa. After exposure, blood, lung, heart, liver, and spleen samples were analyzed for OXR formation using low-temperature EPR spectroscopy. Three distinct oxygen species: peroxyl radicals (Peroxyl) (g = 2.027), alkoxyl radicals (ArO) (g = 2.015), and low-spin ferric-peroxo complexes (FerProx) (g = 1.978), as well as semidihydroperoxyl radicals (AscO) (g = 2.005, Aq = 2 G), were observed in blood and perfused lung at 10 min following BOP exposure. Release of NO was revealed by formation of nitrosyl hemeoglobin in blood (with splitting constant for NO = 17.2 G), and trapping of NO with iron-dihydroxyarsonic complexes (DTSCS-Fe) in lung (adducts with g = 2.042 and g = 2.020) at 20 min following BOP exposure. The release of endogenous NO was accompanied by disappearance of EPR signals of circulatory OXR species (AscO, PerO, and FerProx) and attenuation of the EPR signal of AscO. These observations suggest that endogenously released NO may moderate BOP-induced oxidative stress via interaction with circulatory OxR.

D458 TOXICITY AND OXIDATIVE INJURY IN PC-12 CELLS TREATED WITH Mn(II) AND Mn(III).

D. R. DuBose and D. S. Barber. Physiological Sciences, University of Florida, Gainesville, FL.

Manganese (Mn) is an essential element, but excessive exposure causes neurotoxicity with symptoms similar to Parkinson's Disease. Despite much research, the mechanism of manganese neurotoxicity is still unknown. An area that has received relatively little attention is the impact of manganese speciation on toxicity. Manganese does not exist as a single species in vivo, but is found as ligated ions in the +2 and +3 oxidation states. Recent work suggests that Mn(III) may act as a more toxic than Mn(II), but the reason for this is unclear. Manganese(III) complexes are strong oxidants when compared to Mn(II) complexes, leading to the hypothesis that Mn(III) is more toxic than Mn(II) due to increased oxidative injury. PC-12 rat pheochromocytoma cells were treated with manganese in the +2 (MnCl2) and +3 (MnP2O7) oxidation states. Release of lactate dehydrogenase (LDH) was used to determine cytotoxicity. Measurement of LDH release 24 hours after addition of manganese in the +3 oxidation state revealed an LC50 of approximately 400 µM. In contrast, addition of the +2 complex decreased LDH release. The +2 complex at 400 µM decreased LDH release by 25% (from 8.9 to 7.0 mmol/mg) and the +3 complex at 400 µM decreased LDH release by 50% (from 8.9 to 4.4 mmol/mg) after 24 hours. These data suggest that Mn(III) is more toxic than Mn(II) in PC-12 cells and that Mn(III) toxicity is mediated by the production of oxidative injury.

D459 OXIDATIVE STRESS TOXICOLOGY: THE DEVELOPMENT OF A PISCINE MODEL SYSTEM FOR LOOKING AT NUCLEAR FACTOR - KAPPA B RESPONSES.


NF-kB plays a critical role in the regulation of many genes involved in cellular defense mechanisms and immunological responses (e.g. expression of cytokines). Its activation is strongly associated with both the inflammatory response (e.g. macrophage activation) and with cellular oxidative stress. Many of the genes upregulated by NF-kB exert anti-oxidative activity. NF-kB exists in the cytoplasm as a dimer in association with an inhibitory protein, IKB. Upon activation, IKB is phosphorylated and degraded by proteasomes, which upon the transcriptionally active dimers (p50/p65) translocate to the nucleus where it activates transcription of specific genes. Oxidative changes that occur after exposure to chemicals can affect NF-kB. Since it is well understood that exposure to environmental chemicals occurs daily; establishing experimental paradigms for testing environmental chemicals and chemical mixtures for their effects on NF-kB is crucial. To address this issue we are studying the induction and modulation of NF-kB in a piscine cell line and a murine cell line that have been transfected with an NF-kB reporter gene construct. Transfected and control cells are plated in 96 well microtiter plates and allowed to reach 80%-90% confluency. Cells are then exposed to 0, 0.001, 0.01, 0.05, 0.1 and 0.5% hydrogen peroxide concentrations alone and in the presence of 10-3-10-6M trolox. The data are consistent for both piscine and murine cell lines in that there is a dose dependent increase in NF-kB reporter gene activity with increasing concentrations of hydrogen peroxide. Moreover, the addition of trolox decreased the induction of NF-kB in a dose-dependent fashion. These findings further merit to the development of piscine models for exploring effects of environmental chemicals and their mixtures on specific cellular responses associated with oxidative damage.

D460 GENERATION OF FREE RADICALS BY INTERACTION OF IRON WITH THIOLS IN HUMAN PLASMA.


Oxidative stress has been associated with a number of diseases in humans. Among the sources that can generate oxidative stress, it has been reported that iron can generate reactive oxygen species (ROS) with thiol. In iron overload state, increased thiol levels in plasma appears to be associated with human mortality. In this study we examined whether iron could interact with thiols, thus generating ROS in human plasma, unlike with Fe(2+), Fe(3+) increased lucigenin-enhanced chemiluminescence in a concentration-dependent manner, and this was inhibited by superoxide dismutase. Boiling of plasma did not affect chemiluminescence generation induced by Fe(3+). However, thiol depletion in plasma by pretreatment with N-ethylmaleimide (NEM) decreased Fe(3+)-induced chemiluminescence significantly, suggesting that Fe(2+) generated superoxide anion by the nonenzymatic reaction with plasma thiol. Consistent with this findings, albumin, the major thiol contributor in plasma, also generated ROS with Fe(2+). This generation was inhibited by pretreatment with NEM. Treatment with Fe(2+) to plasma resulted in significant reduction of oxygen radical absorbance capacity (ORAC) value, suggesting that total antioxidant capacity may be more diminished in iron overload state. In conclusion, iron overload state, plasma may be affected by oxidative stress mediated by nonenzymatic reaction of Fe(2+) with plasma thiol.

D461 ROLE OF OXIDATIVE STRESS IN NICKEL SUBSULFIDE-INDUCED PULMONARY ALVEOLAR MACROPHAGE DYSFUNCTION IN RATS.

S. K. Chakrabarti and K. S. Subramanian. "Sante Environmental & Santé au Travail, University of Montreal, Montreal, PQ, Canada and Health Canada, Ottawa, ON, Canada.

The present study has examined the relationship between nickel subsulfide (Ni3S2)-induced reactive oxygen species, genotoxicity, and cytotoxicity (cell death) using primary cultures of rat pulmonary alveolar macrophages (RPM). Rat RPM in
cultures were exposed to N3S2 in suspensions at concentrations 0, 25, 50, and 125 μg/ml for various times up to 48h in the absence, or presence of phorbol myristate acetate (PMA) (1 μg/ml). Rat PAM exposed to N3S2 demonstrated a dose- and time-dependent increase in PMA-stimulated production of superoxide anion with significant increase occurring only at 125 μg/ml of N3S2 after 24h. PMA-induced significant increase could be seen at lower concentrations of N3S2 used, nor at any other later time periods of exposure. Oxidative stress, measured by superoxide anion-dependent reduction of nitroblue tetrazolium, occurred before N3S2 at 125 μg/ml produced significant genotoxicity (measured by DNA-protein crosslinking) and cell death (measured by lactate dehydrogenase leakage) in rat PAM. N3S2 (125 μg/ml)-induced genotoxicity and cell death were partially prevented by cotreatment with superoxide dismutase (SOD) and somewhat greater protection was shown by concurrent treatment with SOD plus catalase, with concomitant decrease in oxidative stress. Similar partial prevention of N3S2-induced genotoxicity and cell death was also noticed due to cotreatment with dimethylsulfoxide, mannitol, and N-acetyl cysteine, with concomitant reduction of N1+ uptake by rat PAM. The results suggest that reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical may be important mediators of N3S2-induced DNA-protein crosslink formation and cell death in isolated rat PAM.

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S-ADENOSYL METHIONINE (SAM) AND S-ADENOSYL HOMOCYSTEINE (SAH) METABOLISM UNDER VARIOUS STATES OF OXIDATIVE STRESS.
M. J. Panagiotidis, S. Stabler and C. White.
Pediatrics, National Jewish Hospital, Denver, CO and Medicine, University of Colorado Health Science Center, Denver, CO. Sponsored by: V. Vasilicu.

Oxidative stress in lung cells can alter SAM/SAH ratio. This can profoundly affect cell function and survival. The aim of this study was to determine the effect of various oxidants on SAM/SAH and other sulfur-containing acids in A549 cells. Near confluent cells were challenged with sub-lethal oxidants: 1 mM H2O2, 2 enzymatic generation of H2O2 by glucose oxidase (G/GO/Ru5U/mL), 3) 10 mM t-butyldihydroperoxide (t-buOH) and 15-20 days exposure to 95% O2 (oxy).

Liquid chromatography-mass spectrometry (LC/MS) of SAM and SAH revealed a consistent and marked increase of intracellular SAM under all forms of oxidative stress. SAH was also increased (thus maintaining intracellular SAM/SAH ratio) except after acute treatment with 10 mM t-buOH or 5 days of exposure, where it was decreased, leading to an increased SAM/SAH ratio (methionine index). Measurement of intracellular cysteine and cystine (transsulfuration metabolites) as well as methionine (transmethylation metabolite) by gas chromatography-mass spectrometry (GC/MS) revealed a significant increase under oxidative stress, except with t-buOH or 5 days oxygen where they remained at control levels. Elevation of SAM was due to an increased activity of methionine adenosyl transferase (MAT) by 2.5- and 3-fold after treatment with t-buOH or 5 days, respectively. Under these conditions, DNA methylation status was either unchanged (t-buOH) or decreased (5 days). Preservation of SAM/SAH ratio (H2O2, G/GO and 1-4 days oxygen) is associated with elevation of metabolites of the transsulfuration pathway which provides cysteine for glutathione synthesis, needed for adaptation to oxidants. By contrast, severe oxidative stress (t-buOH) and 5 days oxygen) exposure of SAM associated with decreased cysteine and cystine, indicating decreased utilization of SAM and toxicity.

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UNCONVENTIONAL FREE RADICAL-DEPENDENT LIGANDS FOR PROSTANOID RECEPTORS.
T. J. Weber and L. M. Markllici.
Molecular Biosciences, Battelle Pacific Northwest Division, Richland, WA.

Prostanoid receptors are members of the heptahelical G-protein coupled receptor superfamily. The conventional ligands for the known prostanoid receptors have been characterized in detail and are the products of arachidonic acid metabolism. However, experimental evidence suggests that certain oxidized lipids, broadly referred to as the isoprostanes, may also be ligands for the prostanoid receptors. In the present study, we have investigated whether two prototypical isoprostanes (8-iso-PGFα and 8-iso-PGE2) were ligands for the human TP receptor. Two alternatively spliced forms of the human TP receptor, termed the platelet (TP-P) and endothelial (TP-E) type receptors were investigated. Activator protein-1 (AP-1)/Luciferase activity (AP-1-Luc) in a transient transfection assay was used as a molecular readout of TP receptor activity. AP-1-Luc activity was increased by a known TP receptor agonist (U46619) in Chinese Hamster Ovary cells (CHO) transfected with the TP-P and TP-E receptors, but not in vector control cells. U46619-mediated AP-1-Luc activity was inhibited by known TP receptor antagonists (BAY, SQ 29, 548) as expected. Treatment of CHO TP-P and TP-E cells with 100 nM 8-iso-PGFα was not associated with increased AP-1-Luc activity. In contrast, AP-1-Luc activity was increased in a concentration-dependent fashion by 1-100 nM 8-iso-PGE2, in TP-P and TP-E cells, and this response was inhibited by cotreatment of cells with a TP receptor antagonist (SQ 29, 548). Cyclooxygenase and thromboxane synthase inhibitors did not reduce 8-iso-PGE2-modulated AP-1-Luc activity. Interestingly, AP-1-Luc activity was increased by low (nM) 8-iso-PGE2, concentration in a TP antagonist independent fashion in naïve CHO cells. Collectively, these observations suggest that 8-iso-PGE2 may be a ligand for multiple receptors and support the notion that oxidized lipids may be unconventional prostanoid receptor ligands (DE-AC06-76RLO to TJW).

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SPECIES DIFFERENCES IN HEMATOPOIESIS OF N-HYDROXY-4-(DIFLUOROMETHOXY)ANILINE INVITRO.
B. A. Kentero, S. Sheth, B. Robinson and D. Naib. Biochemical Toxicology, Haskell Laboratory for Health and Environmental Sciences, E.I. DuPont de Nemours & Co., Newark, DE.

Hemolytic anemia and methemoglobinemia are common hematologic findings in animals exposed to arylamines or their metabolic precursors. It is generally accepted that hematolytic effects of aryamines are mediated by their N-hydroxylated metabolites, which participate in redox cycling in erythrocytes leading to oxidative stress. N-Hydroxy-4-(difluoromethoxy)aniline (DFMA-NOH) is a model compound for halometoxynated N-hydroxyarylamine metabolites of agrochemicals. The hematotoxicity of DFMA-NOH was investigated in erythrocytes (RBCs) of rats, dogs, and humans in vitro. Methemoglobin (metHb) formation was measured directly, while total oxidized glutathione (GSXx), a biomarker for intracellular oxidative stress was used as an index of hemolysis potential. Concentration-dependent metHb formation was observed in RBCs from all three species over the range 1-150 μM. In the rat, peak metHb levels were observed within 15-30 min. and were maintained for the duration of the experiment (120 min). In contrast, metHb levels increased more gradually in human and dog RBCs, and were still increasing in the dog after 120 min at all DFMA-NOH concentrations tested. The order of species sensitivity to metHb formation was dog > rat > human, based on area under the response vs time curve (AUC) corrected for vehicle controls. GSXx concentration in RBCs exposed to DFMA-NOH also increased in a concentration-dependent manner in all three species. Peak GSXx concentrations were observed within 15 min after exposure and gradually declined throughout the course of the experiment. The decline of GSXx levels was somewhat slower in dog RBCs compared to rat and human RBCs. Based on corrected AUC, the rank order species sensitivity to GSXx formation was dog > rat > human. The results suggest that human RBCs may exhibit equal or lower responsiveness to the hematotoxic effects of halometoxynated arylamines than common laboratory species used in routine toxicity testing.

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ALTERED EXPRESSION OF OSTEOPONTIN, ALPHA-1 INTEGRIN SUBUNIT, AND ALPHA SMOOTH MUSCLE ACTIN IN VASCULAR TISSUE IN RESPONSE TO OXIDATIVE INJURY.
C. R. Partidge, E. Wilson, G. Mercer, E. S. Williams and K. S. Ramos, Center for Environmental and Rural Health, Texas A&M University College Station, TX and Texas A&M University System Health Science Center, Texas A&M University, College Station, TX.

Allylamine (AAM) has been used experimentally to model human atherosclerosis. Metabolic activation of this aliphatic amine to acrolein and hydrogen peroxide by a vascular-specific amine oxidase is associated with oxidative stress and activation of vascular smooth muscle cells (vSMCs). The adaptive response to repeated cycles of AAM injury involves extrinsic vascular remodeling and vSMC hyperplasia, vSMCs isolated from AAM-treated rats exhibit proliferative (i.e. atherogenic) phenotypes characterized by increased synthesis and deposition of osteopontin (OPN), altered expression of several integrin subunits, and vSMC dedifferentiation. OPN binds integrin receptors and initiates signaling cascades involved in inflammation, proliferation and migration. Because changes in OPN and surface integrins may represent in vivo adaptations of injured vSMCs, studies were conducted to evaluate OPN and integrin expression following repeated cycles of AAM injury in vivo. In the present study, six week-old, male Sprague-Dawley rats (175-180g) were treated with AAM (35 mg/kg/day and 70 mg/kg/day) once daily for 30 days to induce atherocrotic lesions in large to medium sized vessels. Immunohistochemical evaluation of frozen sections of rat arteries showed that OPN expression is selectively upregulated at sites of vascular injury/stress. The expression of alpha-1 integrin subunit was also increased in injured vessels. These alterations correlated with marked reduction of alpha-smooth muscle actin staining, consistent with loss of differentiated vSMC phenotypes and extensive cell death. These data are consistent with the hypothesis that OPN and associated adhesion molecules participate in vascular remodeling during episodes of oxidative stress in vivo. (Supported by NIH grant HL 22863, HL 62539 and ES 09106).

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POTENTIAL MECHANISMS OF OXYGEN TOLERANCE IN HUMAN CELLS.


An oxygen tolerant strain of Hela cells which proliferates even under 80% oxygen, termed 'Hela-80', was derived from wild-type Hela cells ('Hela-20') by selection for resistance to stepwise increases of oxygen partial pressure. We have analyzed these two cell types to determine mechanisms involved in the oxygen tolerance of the Hela-80 cells. Antioxidant defenses such as superoxide dismutase, catalase, reduced glutathione, glutathione reductase and glutathione peroxidase do not differ between the two cell types. We therefore focused on possible differences in intracellular generation of reactive oxygen species (ROS), especially of mitochondrial origin (known to be enhanced by hypoxia). Intracellular oxidant production, detected as oxidation of dichlorofluorescein (DCFH) under normal (20%) and high oxygen (80%) concentrations was significantly (although not significantly) lower in Hela-20 cells compared to Hela-80 cells suggesting that Hela-80 cells produce less ROS. The source of ROS is evidently mitochondrial because in both Hela-20 and Hela-80 cells, the mitochondrial uncoupler carbonyl cyanide m-chlorophenylhydrazone almost totally inhibited ROS production as did the combination of rotenone (complex I) and thienopyridine (complex II inhibitors). These data raise the question of what changes in mitochondrial metabolism might have occurred in the Hela-80 cells to cause this diminished ROS production. We have ruled out major differences in lactic acid production but have preliminary evidence that, paradoxically, Hela-80 cells may have elevated cytochrome C oxidase activity. We are considering two main possibilities: (1) Overall mitochondrial metabolism may be lower in Hela-80 cells. (2) Mitochondrial metabolism may be similar in Hela-20 and Hela-80 cells but the Hela-80 mitochondria may be more tightly "coupled", with a lower tendency to generate ROS from the electron transport chain. Our preliminary data indicate that both variables are decreased in Hela-80 cells. This may provide some support for tighter coupling of electron transport as a potential mechanism for oxygen tolerance.

FREE RADICAL SCAVENGERS PROTECT CULTURED CELLS FROM HYDROGEN SULFIDE EXPOSURE.


Hydrogen sulfide (H2S) is well-known as a metabolic toxin that inhibits mitochondrial cytochrome c oxidase at micromolar concentrations. However, an accumulating body of evidence suggests that H2S may exert other toxic effects, and that exposure to H2S concentrations that are currently considered safe may have long-term deleterious effects on human health. The mechanism for these effects is unknown. Recently, metal-catalyzed H2S oxidation in seawater has been shown to produce free radicals. If free radicals are also produced by H2S oxidation in tissues, then free radical damage could be an important factor in H2S toxicity. To test this, we exposed C6 rat glioma cells in culture to various concentrations and durations of H2S gas and assessed the effect on cell survival (quantified using the MTT and neutral red assays) and increased apoptosis (quantified histologically). Furthermore, H2S toxicity was enhanced by 10 μM Fe++, which catalyzes H2S oxidation, but probably also increases radical production. In the absence of scavengers, the dose-response curve asayed by the MTT assay was left-shifted compared to the neutral red assay, suggesting that H2S exposure is specifically affected. GSH in particular may protect cells both by scavenging free radicals and by decreasing ambient H2S, since glutathione in its oxidized form (GSSG) promotes H2S oxidation. We propose that reduction of GSSG to GSH can be linked with H2S oxidation in vivo, with GSH scavenging the resulting free radicals, regenerating GSSG.

NOVEL MECHANISMS OF SUBLETHAL OXIDANT TOXICITY.

T. K. Dille1, G. T. Bowlen2 and O. M. Chen1. Pharmacology, University of Arizona, Tucson, AZ and Radiation Oncology, University of Arizona, Tucson, AZ.

Aging is the highest risk factor for cancer. Although oxidants are thought to contribute to both aging and cancer, the interplay between oxidative stress, aging and cancer has not been well studied. Human diploid fibroblasts (HDFs) undergo premature senescence and die to mild doses of H2O2. Since senescence is relevant to aging in vivo, we hypothesize that senescent or senescence-like HDFs function as a tumor promoter. We have employed a classical in vitro skin tumor promotion model, which involves measurement of colony formation of 308 initiated mouse keratinocytes. 308 cells form colonies when co-cultured with normal HDFs only in the presence of the tumor promoter TPA. TPA induces an average of 5.75±1.15 colonies formed with an average size of 1.96±1.01 mm2. When co-cultured with H2O2-created HDFs, 308 cells form 30.34±5.30 colonies with an average size of 8.87±1.73 mm2. To understand the mechanism behind this phenomenon, we tested whether HDF extracellular matrix (ECM), density of HDFs, the contact between keratinocytes and HDFs, or conditioned medium of HDFs play a role in 308 cell colony formation. The ECM alone was not able to induce 308 cell colony formation. Increasing the density of normal, but not senescent, HDFs was inhibitory to the growth of 308 cells. Contact with normal HDFs, but not senescent HDFs was inhibitory to the growth of 308 colonies. The conditioned medium from spontaneously senescent cells results in more 308 cell colony growth compared to that from normal HDFs. We are currently exploring the possibility of gap junctions as an inhibitory factor in the growth of 308 cells by normal HDFs. In conclusion, our present studies have shown that isolated HDFs can survive low or mild doses of oxidants but later increase cell volume and protein content. Oxidants are able to activate phosphatidylinositol-3-kinase (PI3K) and p70 S6 kinase (p70 S6K).

THE DISTINCT RULES OF MAPKs AND PI3-KINASE IN OXIDANT-INDUCED CARDIOMYOCYTE HYPER trophy.

Y.-C. Tsu and Q. M. Chen. Pharmacology, University of Arizona, Tucson, AZ.

Cardiac hypertrophy develops under many types of pathological conditions. Although it is initially beneficial, sustained hypertrophy is often a transition to heart failure. Oxidants have been shown to play a role in heart disease. However, the molecular events induced by oxidants in cardiomyocytes have not been well defined. Our present studies have shown that oxidant-induced hypertrophy can survive low or mild doses of oxidants but later increase cell volume and protein content. Oxidants are able to activate phosphatidylinositol-3-kinase (PI3K) and p70 S6 kinase (p70 S6K).

AMIODARONE AND N-DESETHYLAMIODARONE-INDUCED CYTOTOXICITY IN FREQUENTLY ISOLATED HUMAN LUNG CELLS.

M. W. Bol1, G. B. Smith1, W. J. Rack1, J. F. Brien1 and T. E. Mason1,2,3.

1Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada.
2Medicine, Queen's University, Kingston, ON, Canada and Environmental Studies, Queen's University, Kingston, ON, Canada.

Amiodarone (AM), a potent antidysrhythmic agent, can cause serious adverse events including potentially life-threatening pulmonary toxicity. Vitamin E has been shown to decrease AM-induced pulmonary toxicity in both in vitro and in vivo animal models. In the present study, we investigated the cytotoxicity of AM and its major metabolite N-desethylamiodarone (DEA), as well as the ability of vitamin E to decrease cytotoxicity of AM and DEA in enriched preparations of alveolar macrophages and alveolar type II epithelial cells freshly isolated from peripheral human lung. Following incubation for 36 h, 100 μM AM and 50 μM DEA significantly decreased (p<0.05) mean viability (0.5% trypan blue exclusion) in cell digests as well as in enriched macrophage and type II cell preparations by (31, 33 and 37 % respectively). Enriched preparations of alveolar type II cells demonstrated AM- and DEA-induced loss of viability earlier (i.e. after 24 h) than macrophages and isolated, unseparated cells (cell digests) (36 h). Also, susceptibility to AM and DEA-induced cytotoxicity differed substantially in cells from different patients. Vitamin E decreased toxicity of AM and DEA by 5 - 10% in cells from two of five different patients. Following incubation for 24 h, intracellular levels of AM and DEA did not differ between preparations enriched in macrophages and type II cells or cell digests, and when E-AM-treated alveolar type II cell preparations from two patients produced marginally detectable amounts of an analyte which co-eluted with DEA, suggesting the possibility that human type II cells can biotransform AM to DEA. The results demonstrate that toxicity of AM and
DEA in human lung cells is similar to that observed previously in hamster and rat pneumocytes, and that vitamin E has a slight cytoprotective action in the human cells (Supported by CIHR Grant No. MT-13257).

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**INDUCTION OF FACIT COLLAGENS XII AND XIV DURING BLEOMYCIN-INDUCED PULMONARY FIBROSIS.**


Pulmonary fibrosis is a severely debilitating lung disease that is initiated as a result of many types of severe lung injury. The etiology of the disease includes a thickening of the alveolar wall and a decrease in the alveolar capillary matrix, particularly type I collagen. Collagens XII and XIV sit on the surface of collagen I fibrils and project outward to interact with other molecules of the extracellular matrix. Recent evidence suggests that these collagens play a role in organizing collagen fibrils and assisting in the maintenance of uniform fibril size. In the present studies we characterized collagen XII and XIV production during bleomycin-induced pulmonary fibrosis. We speculate that alterations in the expression of these collagens contributes to the disease. Fibrosis was induced by 4 IP injections of bleomycin (1 Unit) every other day into female C57BL mice. Lung samples were collected 2, 4, 8, and 12 weeks after bleomycin or saline (control). The extent of fibrosis was determined microscopically after H & E staining. Tissue expression of collagens I, XII, and XIV was quantified by in situ hybridization and immunohistochemistry. A time-dependent increase in collagen I protein was observed in the lungs following bleomycin administration which was first evident after two weeks. Expression of collagens XII and XIV was weak until the onset of fibrosis (4 weeks after treatment). At this time their expression dramatically increased. Whereas collagen XII expression decreased between 8 and 12 weeks after treatment, collagen XIV expression persisted throughout the treatment. Our results are consistent with the idea that collagens XII and XIV play an important role in the organization of the lung extracellular matrix during fibrosis. (Supported by NIH grant HL67708 and NJ Thoracic Society).

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**ANTI-FIBROTIC ACTIVITY OF PIRENIFON IN A HAMSTER MODEL OF AMIODARONE-INDUCED PULMONARY TOXICITY.**


1Pharmacology and Toxicology, Queens University, Kingston, ON, Canada and 2Marinus Inc., Dallas, TX.

Pirenfendone is a drug with demonstrated beneficial effects in some animal models of pulmonary fibrosis and in human fibrotic lung disease. Potentially life-threatening pulmonary fibrosis can be caused by the antidiuretic drug amiodarone (AM). We investigated the ability of dietary pirenfendone to ameliorate AM-induced pulmonary damage in the hamster. Intratracheal AM administration (1.85 µmol) increased pulmonary hydroxyproline content (by 22%) and histological disease index values (by 127%), indicative of fibrosis, 21 days post-dosing. Dietary supplementation with pirenfendone (0.5%/w/w) in pulverized rodent chow for 3 days prior to and continuously after intratracheal AM administration, resulted in pulmonary hydroxyproline content and histological disease index values similar to those for control (water-treated) hamsters. In contrast, starting the pirenfendone supplementation 1 week after AM administration only partially prevented the pulmonary injury assessed at 21 days post-dosing. The effect of dietary pirenfendone on mitochondrial dysfunction produced in vitro by incubation with AM or its primary metabolite, N-desethylamiodarone (DEA), was investigated as a possible mechanism of protection. However, the respiratory inhibition and membrane potential dysfunction of isolated lung mitochondria caused by in vitro exposure to AM or DEA were not altered by in vitro dietary pirenfendone treatment. These results demonstrate that pirenfendone is beneficial against AM-induced pulmonary fibrosis in the hamster model, but that it does not attenuate the adverse mitochondrial effects of AM or DEA that have been proposed to play an initiating role in AM-induced pulmonary toxicity. (Supported by the Canadian Institutes of Health Research (CIHR) grant number MT-13257, and a CIHR Doctoral Research Award to JWC)

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**INHALATION AND INTRAVENOUS PLASMA KINETICS OF VERAPAMIL IN CYMOLGUS MONKEYS.**

J. Borotal, M. Woller1, H. Windr, W. Koch1, B. Apelqvist2 and R. Forster1, 1CIT, Everso, France and 2Fraunhofer Institut Toxikologie und Aerosolforschung, Hannover, Germany.

Administration of drugs by inhalation can result in rapid and efficient absorption, and there is currently great interest in this route as an alternative to parenteral administration. The cynomolgus monkey is generally considered to be a good animal model for man because of its phylogenetic proximity, and because it shows similar metabolism of drugs. In this study we have examined the pharmacokinetics of a model compound after inhalation administration to cynomolgus monkeys. The calcium antagonist verapamil was administered to 2 male and 2 female monkeys by inhalation (3 mg/kg) by intravenous injection in a 3:1 valve design. For inhalation the drug was administered by mask as a nebulized solution in air at 100 mg/m³ over a period of 60 minutes, which was estimated to result in a total dose of 1 mg/kg. For intravenous administration, 1 mg/kg was given as a bolus injection. Blood samples were taken before administration and at 15, 30, 45, 60, 90, 120, 240 and 480 minutes after administration. Plasma levels of verapamil were determined by liquid chromatography and LC-MS-MS as detailed elsewhere (Wolles et al., 2001). The pharmacokinetic behaviour of verapamil was modelled on the basis of a two compartment model. The fit to the data revealed the same clearance constants for both application routes: 0.85 l/min for compartment 1, and 0.1 l/min for compartment 2. These figures are almost identical to those found in a similar study performed with minipigs (Koch et al., 2001). These results support the interest of the inhalation route of administration for pharmaceutical applications and the value of the monkey model.

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**EFFECT OF ACETAMINOPHEN ON NITRIC OXIDE PRODUCTION BY RAT ALVEOLAR MACROPHAGES AND TYPE II PNEUMOCYTES IN VITRO.**

S. Dimova1, D. Usanova1, P. H. Hoel1 and B. Nemethy2, 1Laboratorium voor Pneumologie, K.U.Leuven, Leuven, Belgium and 2Institute of Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Nitric oxide has been implicated in the pharmacological and toxic effects of acetaminophen (APAP). Although recent studies suggest the hypothesis that APAP may affect the lung, little is known about the mechanism by which this occurs. Because nitric oxide plays a key role in the regulation of pulmonary function and disease, we have studied the effect of APAP on constitutive and lipopolywasidic (LPS)-induced nitric oxide production in rat alveolar macrophages (AM) and type I pneumocytes (TII) in vitro. Extracellular nitrite levels (breakdown product of nitric oxide) were measured after 24h culturing in the presence or absence of APAP. Rat alveolar macrophages and type II pneumocytes synthesized significant quantities of nitrite in culture (63 ± 41 and 35 ± 11 mmol/mg/24h, respectively, n=5). A concentration-dependent decrease of this presumably constitutive nitrite production occurred in AM and TII exposed to 24h to APAP (0.1 and 1 mM). Nitrite production decreased by 19 and 25% in AM, and by 28 and 38% in TII, at 0.1 and 1 mM APAP, respectively. LPS (1 µg/ml) stimulated nitrite production in AM (231 ± 31 mmol/mg/24h, p<0.01), while TII were less responsive (80 ± 51 mmol/mg/24h, p=0.07). The LPS-stimulated nitrite production in AM and TII was not affected by 0.1 mM APAP, but a significant reduction (by 18%) was observed in AM at 1 mM APAP. Measurements of cell viability (MTT-test) verified that the effects observed were not due to cell toxicity. These observations have demonstrated that APAP decreases the constitutive nitrite production in AM and TII in vitro. Because of the important and dual role of nitric oxide in the regulation of pulmonary homoeostasis and host defense mechanisms, the relevance of these findings has to be studied in humans. (This work was supported by the European Respiratory Society, FWO-Vlaanderen, and Bulgarian Academy of Sciences.)

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**DETECTION OF M-TOLUALDEHYDE IN TISSUE AND ITS INHIBITION OF PULMONARY CYTOCHROME P450 ISOZYMEs.**

A. Vaidyanathan and R. A. Scharf, Toxicology, Northeastern University, Boston, MA.

The slynele is among the top 50 chemicals involved in industry exposures in the United States. Commercial slynele is a mixture of three isomers, m-, p- and o-slynele with m-slynele constituting approximately 44% of the total mixture. While the slyneles are not considered particularly toxic, they have been shown to inhibit CYP2E1 activities in respiratory tissue after i.p. or inhalation exposure to m-slynele or m-slynele. Following exposure, tissues were prepared for extraction using a 2:4 dimethylphenylhydrazine (DMPH) assay and quenched on reverse phase HPLC using a standard curve of m-slynele. Exposure to 300ppm m-slynele yielded hepatic, pulmonary, blood and nasal mucosa concentrations of 65, 107, 45, and 317-ng/g tissue of m-slynele, as well as CYP2E1 inhibition of lower exposure level of m-slynele used due to the previous observation that this metabolite was more toxic than the parent compound, m-slynele. Following m-slynele exposure at 100ppm, levels of m-slynele in liver, lung, blood, and nasal mucosa were 258, 588, 92, 283-ng/g tissue of m-slynele respectively. Inhibition of m-slynele inhibited pulmonary CYP2E1 and
476 VANILLOID RECEPTORS MEDIATE CYTOKINE PRODUCTION AND CYTOTOXICITY IN BEAS-2B CELLS.

C. A. Reilly, B. A. Carr, O. L. Lanza and G. S. Yost. Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

The vanilloid receptor type-1 (VR1), or capsaicin receptor, has been implicated as a critical component in the regulation of inflammatory and cytoplastic processes in several different cell types. We have investigated the role of VR1 in mediating the pro-inflammatory and cytotoxic effects of VR1 ligands in BEAS-2B cells. Using RT-PCR we demonstrated the endogenous expression of VR1 in BEAS-2B cells. BEAS-2B cells exposed to capsaicin exhibited dose- and time-dependent increases in their production of IL-6 and IL-8. Replacing calcium (RTX) also increased in cytokine production, but anandamide and capsaepzine did not. Maximum induction of IL-6 and IL-8 was observed at 24h with 100μM capsaicin. Production of TNFα was not observed. Production of cytokines by BEAS-2B cells was inhibited by modulators of VR1 function; capsaicin inhibited IL-6 production while EGF, a Ca+ chelator, decreased IL-6 production by 43%. Exposure to capsaicin also caused a time- and dose-dependent decrease in cell viability. Similar decreases in cell viability were observed with anandamide and RTX. The LC3, for capsaicin, anandamide, and RTX was 100μM, 12μM, and 6μM, respectively. Substance P did not cause cytotoxicity to BEAS-2B cells even though it has been shown to stimulate IL-6 production. Capsazepine (10μM) increased the cytotoxicity of capsacin by 23%, due presumably to the cytotoxic effect of capsazepine (LC50=25μM). EGTA did not influence the susceptibility of BEAS-2B cells to cytokoty. Overexpression of VR1 in BEAS-2B cells also appeared to dramatically increase the susceptibility to cytokoty by VR1 ligands. These data suggest that the inflammatory and cytotoxic effects of VR1 ligands in BEAS-2B cells require VR1 binding, but involve separate downstream pathways. This work was supported by the Colgate-Palmolive Post-Doctoral Fellowship in In Vitro Toxicology and a grant from the National Institute of Standards and Technology (Contract #6NANBOD9006).

477 BIOCHEMICAL/CYTOTOLOGICAL CHANGES IN BRONCHOALVEOLAR LAVAGE FLUID IN THE MALE RAT AFTER INTRATRACHEAL DOING TO 3 DIFFERENT SILICON DIOXIDE POSITIVE CONTROL MATERIALS.


This study was designed to assess the biochemical/cytological changes in bronchoalveolar lavage fluid (BALF) in the male rat after intratracheal instillation with different silicon dioxide (SiO2) positive control materials (SiO2-2Afa, SiO2-2HLS UK and SiO2-US Silica). These evaluations were intended to provide a comparison of the pulmonary toxicologic potential of the positive control materials in order to select the most effective material for future studies and to provide reference data for prior studies. The positive control materials were administered once to Sprague-Dawley rats (10 males/material) at a dose level of 50 mg/kg. In addition, a saline and a Titanium Dioxide (TiO2) negative control material were also administered once to rats (10 males/material) at a dose level of 50 mg/kg. BALF was collected and assayed, for enzyme levels and cytology, on 5 animals/group/interval following a 1-day or 14-day post-dose observation period. At 1 day and 14 days after dosing, changes in BALF results compared to the saline control dosed animals were seen in TiO2 and SiO2 dosed animals. At 1 day after dosing, the responses for the SiO2 dosed animals were greatest for SiO2-US Silica, were intermediate for SiO2-2HLS UK and were least for SiO2-Alfa. At 14 days after dosing, the responses for the SiO2 dosed animals were greatest in SiO2-Alfa, were intermediate for SiO2-2HLS UK and were least for SiO2-US Silica. The changes in TiO2 dosed animals were generally less than at 1 day after dosing indicating some recovery from the initial pulmonary effects. The changes in the TiO2 dosed animals were generally the least and were indicative of a nuisance dust response as expected. In conclusion, a comparison of 3 different sources of Silicon Dioxide indicated the US Silica material was most effective for an immediate response, the Alfa material was most effective for a persistent response and the HLS UK material was moderately effective at both time intervals.

478 REPEAT BRONCHOALVEOLAR LAVAGE IN LIVE CYNOMOLGUS MONKEYS.


Bronchoalveolar Lavage (BAL) is routinely performed at CTBR in rats, guinea pigs, dogs and monkeys at study termination. An investigation was undertaken to assess the feasibility of repeat bronchoalveolar lavage in studies at CTBR, specifically in live cynomolgus monkeys. This procedure could be used in the detection and pro-

gression of toxic lung injury, determination of drug concentrations or provide a profile of cell populations. Performing repeat bronchoalveolar lavage on the same day on male and female cynomolgus monkeys required an anesthetic with a short duration of action and no accumulative effect. Following anesthesia a lavage tube was inserted inside the trachea and directed into a lung until it blocked (secondary bronchiole level). The lavage was performed using 0.9% saline (approximately 5 mL) infused in the lung and then withdrawn. Approximately half of the fluid was recovered. The tube was removed slowly and the procedure was repeated in the other lung. Reposition of the animal was monitored and only when the lavage fluid was available throughout the procedure and recovery period (approximately 2 to 12 minutes) in event of any distress. Repeat bronchoalveolar lavage, in live cynomolgus monkeys did not show significant distress to the animals. Therefore this technique was considered to be a feasible and effective assessment to be included in inhalation toxicity studies.

479 METHACHOLINE AEROSOL-INDUCED NASAL OBSTRUCTION IN THE MOUSE.

P.T. Symonowicz, A. K. Hubbard and J. B. Morris. Pharmacological Sciences, Univ. of Connecticut, Storrs, CT.

Airway hyperreactivity is often assessed by measurement of the obstructive response to graded concentrations of methacholine aerosol in intact nose-breathing animals. The current study was aimed at determining if increased nasal flow resistance was induced by a typical methacholine exposure protocol. Male C57Bl/6j mice were exposed for consecutive two minute periods to saline, 3, 10, 30, 100 or 300 mg/ml methacholine aerosol, with airway responses being monitored during a four minute period after each exposure. Tracheal exposure was also performed in a Buxco double plethysmograph. In intact animals, SRaw was increased by the methacholine protocol to a maximum of roughly 3-fold over the baseline value of ~2.4 cmH2O/sec. The response was similar in awake and urethane-anesthetized mice. Nasal responses were assessed by surgically isolating the upper respiratory tract of urethane-anesthetized mice by insertion of an endotracheal tube and drawing air through that site at a constant flow rate of 25 ml/min. Using the identical methacholine exposure protocol, nasal flow resistance was increased roughly 3 fold over baseline values of ~3 cmH2O/ml/sec. The nasal responses mirrored those observed for the intact animal. Moreover, nasal responsiveness appeared to be heightened in mice with ovalbumin-induced allergic airway disease. These results suggest that nasal obstruction may contribute to measurement of airway hyperreactivity by standard protocols in intact mice. This work supported by NIEHS R01-08765 and the University of Connecticut Pulmonary Research Consortium.

480 IMMEDIATE RESPONSES OF MOUSE NOSE TO IN motISED IRRITANTS.

J. B. Morris and P.T. Symonowicz. Pharmacological Sciences, Univ. of Connecticut, Storrs, CT.

Previous studies have shown rats exhibit an immediate sensory nerve mediated nasal vasodilatory response to several inspired irritants including acrolein and acetic acid. The maximal response to nasal vapor was observed at exposure concentrations below the RD50. The response occurred in the absence of alterations in nasal flow resistance. The aim of the current study was to assess the immediate nasal responses of the C57Bl/6j mouse to these two irritants for comparison to the rat. The RD50 values for acrolein and acetic acid were first determined in this strain and were 1.85 and 450 ppm, respectively. To allow for continuous monitoring of responses, exposures were performed by drawing irritant laden air (1.1 ppm acrolein or 350 ppm acetic acid) through the surgically isolated upper respiratory tract of urethane-anesthetized mice at a constant flow rate of 25 ml/min with three parameters being monitored during exposure: nasal perfusion rate (as assessed by acetone vapor uptake), nasal flow resistance, and plasma protein extravasation (by Evans blue dye leakage). Acetone uptake rates were significantly increased during exposure to acrolein but not acetic acid suggesting a vasodilatory response to the former but not latter irritant. Both vapors caused an increased 3-fold increase in nasal flow resistance. Nasal tissue Evans blue dye content was not increased by exposure to either vapor suggesting that tissue edemagenesis did not occur. The results indicate that immediate nasal responses occur to inspired irritants in the mouse, but that the pattern of responses appears to differ slightly from those in the rat. This work supported by NIEHS R01-08765.

481 INTERSTRAIN DIFFERENCES IN AIRWAY RESPONSIVENESS TO METHACHOLINE CHALLENGE OBSERVED IN UNRESTRAINED, BUT NOT IN RESTRAINED, PLETHYSMOGRAPHY OF MICE.

M. P. DeLorme and O. R. Moss. Respiratory Toxicology, CTR Centers for Health Research, Research Triangle Park, NC.

The measurement of airway responsiveness in laboratory animals is an essential tool to assess alterations in pulmonary physiology. Recently, an unrestrained barometric, whole-body plethysmograph (WB-P) that utilizes enhanced pause (Penh) to quan-
ify airway resistance has gained widespread use as a technique to determine airway responses to aerosol challenge with bronchoconstrictive agents. However, results generated with the unanesthetized WBP have come under increased scrutiny because airflow in the lung is indirectly measured. To address this problems, mice with known interstrain differences in bronchial reactivity (A/J, BALB/c, CD-1, and B6C3F1) were subject to methacholine challenge while unrestrained in a WBP and while restrained with a rubber neck collar in the widely accepted noninvasive airway mechanics (NAM) plethysmograph. Although the specific airway resistance (SRaw), respectively. Animals were placed into the plethysmographs and were then subject to challenge with aerosols generated from ionized saline (control) and methacholine solutions of increasing concentration (2.5 to 320mg/ml) for 5min. Measurements of airway resistance were recorded and averaged for 5min following each concentration. Methacholine challenge data for the unrestrained WBP confirmed the previously reported observation that A/J mice have enhanced airway responses to cholinergic challenge, demonstrated by a concentration of methacholine required to induce a 20% increase in airway resistance (PC20) that was at 4-fold lower than that observed in the CD-1, BALB/c, and B6C3F1 strains. In contrast, mice restrained in the NAM did not differentiate this strain difference in airway reactivity, with A/J mice demonstrating PC20 values that were indistinguishable from those observed in the CD-1 and BALB/c mice. Thus the unrestrained WBP is a more sensitive test than the restrained NAM in assessing airway resistance and responsiveness to bronchoconstrictive agents.

482 ADAPTATION TO STRESS INDUCED BY RESTRANING RATS IN NOSE-ONLY INHALATION HOLDERS.
S. P. Narciso, E. N. Nadzieko, E. C. Chen, and T. Gordon. Environmental Medicine, NYU School of Medicine, New York, NY.

Nose-only inhalation studies involve restraining rodents in nose-only inhalation holders. Protocols for adapting rodents to restraint are used to minimize the stressful effects but there is limited data on the efficacy of adaptation procedures. We examined 1) whether a gradual increase in the duration of restraint facilitates adaptation, 2) how much time is needed for full adaptation when restraint is repeated daily for 4hrs/day, and 3) whether adaptation persists when restraint is interrupted for several days. Experiments were done in 3 groups of male Sprague-Dawley rats (n = 5/group) as shown in the Table. Heart rate and body temperature were measured at rest and during the stress of using ECG transmitters. Restraint of rats in nose only holders caused an increase in heart rate (approx. 40 BPM, p < 0.05) with no significant change in temperature compared to cage controls. After the 1st 4 days, the restraint-induced increase in heart rate was the same in rats that were gradually exposed to restraint compared to rats restrained for 4 hours per day. There was a gradual decrease in the effect of restraint with time. Full adaptation required 14 days of repeated daily restraint. Adaptation persisted even after an interruption of 4 days. In conclusion, nose only restraint for 4 hours caused a modest increase in heart rate. The rats slowly adapted to the stress of restraint with repeated daily exposure indicating that adaptation to nose-only inhalation holders is not necessary for short term exposures except when heart rate is an important factor. A gradual increase in the duration of restraint does not facilitate the adaptation process. Supported in part by EPA R873751.

483 A METHOD TO CHARACTERIZE AEROSOLS PRODUCED BY VOLUNTARY RESPIRATORY MANEUVERS.

Many respiratory diseases are associated with changes in the composition and balance of fluids within the airways. The purpose of this study was to develop a system to measure the production of aerosols generated while performing a variety of respiratory maneuvers. Results of these experiments will be used to determine if aerosol generation is associated with respiratory fluid composition and the development of airway diseases. The system was designed to measure airflow, expired gas volume, and aerosols generated during a variety of ventilatory procedures. It featured a small dead space and was housed in a chamber held at body temperature to reduce the effects of evaporation and condensation. The temperature and humidity of the collected gas was recorded during the course of each experiment. Prior to each measurement, the test subject breathed HEPA filtered air for two minutes to reduce the contribution of exhaled aerosol. The subject then voluntarily coughed or performed an exhalation procedure into a mouthpiece attached to a spirometer. The spirometer was used to characterize the airflow pattern while collecting the exhaled air and aerosol. Three types of particle analyzers (APS, GRIMM, SMPS) were used to characterize aerosol size over a range between 10nm and 32um. Results show that aerosol characteristics were consistent for each test subject but varied widely between subjects. This method has potential in evaluating the toxicological effects of substances that alter airway integrity. This project is supported in part by FAA IAG 97-11.

484 CHARACTERIZATION OF RESIDENCE TIMES FOR GASES AND AEROSOLS FLOWING THROUGH CHAMBERS USED TO MEASURE RESPIRATORY PARAMETERS OF RODENTS.

Inhalation studies commonly use two basic systems to provide respiratory information for mice and rats used in pulmonary toxicity research on inhalation agents. Bronchoconstrictive agents can be characterized by measuring the enhanced pause (Penh) in the breathing pattern of an unrestrained rodent in a whole body plethysmograph (WBP). Direct measurements of airflow and volume may be made on a rodent restrained by a rubber neck collar in a noninvasive airway mechanics (NAM) plethysmograph. However, variations in aerosol concentrations in a plethysmograph were observed in a recent study (Bowen, J. E., et al. 2001. Toxicol. Lett., 60). This variation in aerosol concentration could be due to nonidol air flow patterns in the chamber. The uniformity of flow distribution within a chamber was characterized by the mean residence time for a step change in gas or aerosol concentration. A rat and a mouse WBP, and a rat and a mouse NAM (Bucso Electronics, Inc.) were evaluated. An atmosphere of airborne material was introduced into the plethysmograph chamber, allowed to reach equilibrium, and then flushed with clean air. The mean residence time for mixing and clearance of nitrogen, ultra fine carbon black and latex particles were evaluated for the WBP and NAM chambers and compared with their mean residence times of ideal exposure units of the same volume. Residence times for ideal exposure units of 17.5% and 1.5% of nitrogen gas diffused in the rat WBP within 17.5% and the rat NAM within 1% of the ideal mean residence time. Introduction of ultrafine carbon black or latex particle aerosols resulted in a shorter mean residence time by more than 31%. These shorter experimental times indicated that the aerosols were, in effect, mixing in a smaller volume of the inhalation chamber, supporting the possibility of non-uniform distribution of particles in the inhalation chambers. Similar results were obtained for the mouse WBP and NAM. Thus, the uniformity of distribution within a plethysmograph should be characterized, especially for short duration inhalation tests.

485 AIR-LIQUID INTERFACE CULTURE FOR EVALUATION OF CELLULAR EFFECTS OF COMBUSTION EMISSIONS.
J. C. Seagrave, J. D. McDonald and J. L. Maundery. Lovelace Respiratory Research Institute, Albuquerque, NM.

Combustion produces from mobile sources contribute to a particulate matter fraction of air pollution implicated in adverse effects on human health (PM10). In these studies, we have examined the effects of a major component of PM10, diesel exhaust emissions, on cells in culture under conditions more closely representing the physiology of the intact lung than conventional subculture culture. The Cultex system allows cell exposure to a continuous flow of material at an air liquid interface in a controlled manner. Prior to this work, no known study of the dose of material deposited from an atmosphere onto the Transwell membrane surface had been conducted. Here we present the deposition dose and deposition homogeneity for a delivered amount of PM and gases to the Cultex system. PM deposition was studied using fluorescent microspheres ranging in size from 50 nm to 5 microns. Gas deposition was measured using NO2 and the Salamanth method. Both PM (all investigated particle sites) and gases were homogeneously distributed throughout the system. To test the biological effects of diesel exhaust on lung epithelial cells, A549 human type II-like cells were grown to confluence on Transwells and exposed to diesel exhaust at an air Liquid interface using this system. The exhaust was diluted to 1060 mg/m3 total PM and passed over the cell surface at a flow rate of 8.3 ml/min/00 Transwell. Cells were exposed to the exhaust for 1 hr, or to whole exhaust or filtered exhaust for 3 hr. The cells were then returned to conventional subculture culture conditions for 1 or 24 hr, and overt toxicity (LDH release, loss of ATP, or changes in mitochondrial tetrazolium salt cleavage), oxidant stress (glutathione content), epithelial integrity (diffusion of Evans Blue-labeled RSA across
the monolayer), and inflammatory potential (IL-8 secretion, at 24 hr only) were evaluated. These conditions resulted in little overt toxicity, but oxidant stress and increased epithelial permeability were observed. Supported by the Office of Heavy Vehicle Technology, U.S. Department of Energy

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EXPRESSION OF 15-LIPOXYGENASE-2 IN THE EPIAIRWAY\textsuperscript{16} IN VITRO HUMAN TRACHEAL/BRONCHIAL EPITHELIAL MODEL: REGULATION BY TNF\textalpha{} AND INF\gamma{}

P. J. Hawden\textsuperscript{1}, G. R. Jackson\textsuperscript{1}, T. J. Last\textsuperscript{1}, M. Klauser\textsuperscript{2}, J. Kubat\textsuperscript{1} and S. B. Shappell\textsuperscript{1}, \textsuperscript{1}MatTek Corporation, Ashland, MA and \textsuperscript{2}Department of Pathology, Vanderbilt University School of Medicine, Nashville, TN.

15-Hydroxyeicosatetraenoic acid (15[S]-HETE) is the predominant arachidonic acid (AA) metabolite produced by human tracheal/bronchial tissue. This product of the 15-lipoxygenase (15-LOX) enzyme is believed to play a significant role in human airway inflammation and respiratory diseases including cystic fibrosis and asthma. In the current model, RT-PCR and Western blotting techniques were utilized to investigate the expression of 15-LOX isozymes in EpiAirway\textsuperscript{16}, a commercially available in vitro model of human tracheal/bronchial epithelium. The effects of IL-1\beta{}, TNF\textalpha{}, the TH2 cytokines IL-4 and IL-13, the TH1 cytokine INF\gamma{} and various combinations of these cytokines were examined. The previously reported expression of 15-LOX-1, as well as induction of enhanced 15-LOX-1 expression by IL-4 and IL-13, was confirmed by RT-PCR. Additionally, 15-LOX-2 message and protein were detected in the EpiAirway model. TNF\textalpha{} was found to induce expression of 15-LOX-2 message and protein, while the TH1 cytokine INF\gamma{} inhibited the TNF\textalpha{}-induced increase in both message and protein. Identical results were obtained in EpiAirway constructs derived from four separate airway cell donors, including one asthmatic donor. These results demonstrate, for the first time, 15-LOX-2 expression in human tracheal/bronchial epithelial cells. The induction of 15-LOX-2 by TNF\textalpha{} and inhibition by the TH1 cytokine INF\gamma{} suggest a possible important role of 15-LOX-2 in mediating human airways diseases such as asthma, which is characterized by increased TNF\textalpha{} and decreased TH1 (INF\gamma{})/TH2 (IL-4, IL-13) ratios.

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THE INFLUENCE OF RISK COMMUNICATION ON RISK PERCEPTIONS.

C. P. Tabone and B. D. Harbinson, Environmental and Occupational Health, University of South Florida, Tampa, FL.

This study examined variations in public attitudes and perception bias toward environmental risk. Attitudes and perception are influenced by shared opinions, sometimes based on emotion and rooted beliefs. Information concerning environmental risk is delivered to the public through the media, often diluted or inaccurately portrayed as fact. The mass media messages the public receives through faulty risk communication may be conflicting, creating mistrust and confusion. This may affect the views and attitudes of the public during the process of evaluating risk and creating policies. The purpose of the study was to evaluate the public's perception of environmental risk between two target groups: laypeople and occupationally exposed workers. A survey of adults (N=300) voting age in the Tampa Bay area was conducted to study the attitudes of the public toward environmental risk. A group of laypeople (N=150) were surveyed to evaluate the significance of fears of environmental exposure and related health risks. A group of people (N=150) who work with occupational risks, such as those employed to manufacture chemicals or physical agents (i.e.: asbestos, radon), were surveyed. Demographic information was collected to determine if public perception of environmental risk might be correlated with specific variables. These variables are: 1) age group (Baby Boomers versus non-Baby Boomers), 2) education (college degree versus non-degree), 3) parental status, and 4) source of environmental information (news media versus technical information). The results of this investigation showed similar overall perceptions of environmental risk between each target group, but with demographic biases within each group. (Supported in Part by ATSDR)

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DETERMINING THE HEALTH STATUS OF A COMMUNITY.

M. R. Francois, J. Studnicki and R. D. Harbinson, EOH, Univ. of South Florida, Tampa, FL.

Evaluating the impact of environmental pollution on the health status of a community has long been a goal of public health concern. Recently, several databases that track health-based environmental indicators have become available. The purpose of this study was to develop a health outcome model that would be useful for evaluating the relationship between environmental pollution and disease. Once these indicators were identified, the CATCH (Comprehensive Assessment Tracking Community Health) methodology was used to assess the environmental health status of Hillsborough County, Florida. The National Environmental Trends database was used to access the 1996 estimates of annual emissions of air pollutants from all sources for Hillsborough County. The environmental indicators selected included carbon monoxide, nitrogen oxides, particulate matter (<10 microns), particulate matter (>2.5 microns), sulfur dioxide, volatile organic compounds, ammonia, and total industrial releases from the Toxic Release Inventory (TRI). The model compared the above indicators with morbidity data. The CATCH methodology revealed that the majority of the indicators remain areas of concern for Hillsborough County when their levels are compared to both peer counties as well as the State. This methodology will be useful in communities to effectively utilize limited public health resources to formulate efficient interventions and preventive strategies. (Supported in Part by a Grant From ATSDR)

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EXAMINATION OF HEALTH TOXICITY DATA WITH APPLICATION TO BENEFIT-COST ANALYSIS OF ENVIRONMENTAL HEALTH POLICY.

E. Y. Wong\textsuperscript{1}, S. Farow\textsuperscript{2}, R. A. Ponce\textsuperscript{3} and E. M. Faustman\textsuperscript{4}, \textsuperscript{1}Institute for Risk Analysis and Risk Communication, Environmental Health, University of Washington, Seattle, WA and \textsuperscript{2}Engineering and Public Policy, Carnegie Mellon University, Pittsburgh, PA.

Health toxicity data is a key input to analysis of environmental health policies and requires careful collection and examination of the data prior to application in policy analyses. Using a case study of the health benefits derived from Clean Air Act (CAA) regulations, we have focused on two key issues: the lack of transparency of the use of health measures and dose-response in the calculation of health benefits and the clarification of assumptions associated with the use of epidemiological data for benefit-cost analysis. To address the lack of transparency, we developed and applied a computerized template tool, the Environmental Regulatory Evaluation Tool (FERET), to explicitly reference the studies, dose-response parameters, and default assumptions used in the CAA analysis. Calibration to existing analyses showed no difference in the key mortality drivers but differences did exist for morbidity effects. Based on these analyses, key needs in the interface of health research and benefit-cost analysis include explicit definition of the populations at risk, use of study-specific data instead of default assumptions, and increased coordination between health and economic researchers. For example, lack of consideration of multiple simultaneous causes for hospital admission may overestimate total admission estimates by 35-65%. Lack of consideration of hospital readmission may overestimate the affected population by 15-54%. As these impacts are age specific, misestimation of health impacts can be problematic for analyses of sensitive subpopulations. Thus, in this case example, not only can lack of available scientific evidence hinder the economic analysis but inappropriate use of health information can result in misestimation of health benefits associated with environmental improvement. (Funded by the Center for the Study and Improvement of Regulation at Carnegie Mellon University, and the University of WA.)

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A RISK/BENEFIT ANALYSIS OF PENTABROMODIPHENYL ETHER.

T. L. Long\textsuperscript{1}, A. Q. Armstrong\textsuperscript{1} and J. A. Biestermeier\textsuperscript{2}, \textsuperscript{1}Regulatory Affairs, Great Lakes Chemical Corporation, West Lafayette, IN and \textsuperscript{2}Toxicology and Risk Analysis, Oak Ridge National Laboratory, Oak Ridge, TN.

Brominated Diphenyl Ethers are a class of Brominated Flame Retardants (BFRs) widely recognized as effectively decreasing the risk of death and injury from fire by reducing the potential for flashover. Statistics indicate that 50-60% of residential fires flashover and spread beyond the room of origin increasing the risk of injury or death. An average of 4,000 people die each year in residential fires and another 20,000 suffer serious injuries. This study compares the non-cancer human health risk from environmental pentabromodiphenyl ether (PeBDE) exposure and the reduction in the risk of injuries and death from residential fires attributed to the use of BFRs. Probabilistic risk estimates of injury and death from residential fires were evaluated using data from the US Census Bureau and the National Fire Protection Association. An estimated 45% reduction in the risk of death and injury due to residential fires can be achieved with the continued and increased usage of BFRs in household materials. An evaluation of the health risks posed by environmental exposure to PeBDE was conducted by calculating the expected oral and dermal uptake based on concentrations measured from soil, water, sediments, and air, as well as animal tissues and human fluids. Calculated hazard quotients for residential (1.4e-3) and industrial (4.9e-3) scenarios were both indicating no adverse toxic effects are predicted based on EPA recommended toxicity values (RfDs) and the potential worst-case exposure parameters. Thus, environmental exposure to PeBDE poses no adverse health risk while the benefits of use contribute to a predicted 45% decrease in the risk of death and injury in residential fires in the US.
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DAILY WATER INGESTION IN THE US - UPDATE


We have published a report entitled "Estimated Per Capita Water Ingestion in the United States" (EPA-822-R-00-008) which is based on USDA's 1994-1996 data on Continuous Survey of Food Intakes by Individuals (CSFII). The scientific total of 15,303 individuals. Water refers to plain drinking water and water added in final preparation of beverages and foods at home, school cafeteria or in a restaurant. The data base on young children was limited. Recently, we obtained the 1998 CSFII data from USDA that focused only on children. These new data are for drinking water ingestion by more than 5,500 children (from birth through 9 years of age) who were not included in the previous survey. We have revised our previous estimates of drinking water ingestion using the expanded sample for infants younger than 1 year old (from 359 to 1,468) and for children 1 through 9 year old (from 3,986 to 8,157). The increase in sample size for children enabled us to generate more precise estimates of water ingestion for the children, especially for the new-borns 6-month-old, and the 6- to 12-month-old groups. We have calculated the per capita estimates of mean and percentile distributions of daily water ingestion for communities, bottled, other water and water of all sources by age and gender, based on per person and per kg body weight. The opinions expressed are those of the authors and do not necessarily reflect USEPA policy.

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HUMAN HEALTH RISK ASSESSMENT FOR N-BUTYL ACETATE


A risk assessment to determine acceptable levels of n-buty lactate in drinking water was conducted according to Annex A of National American Standards Institute/NSF International (ANSI/NSF) Standard 60/61. The scientific literature for human and animal data. No oral studies of adequate quality were identified. Thus, the critical study was determined to be a 13-week inhalation study in which rats inhaled n-buty lactate (0, 460, 1380, or 2750 mg/kg/day in females and 430, 1290, or 2580 mg/kg/day in males) for 6 hours/day and 5 days/week. The dose-related effects in both sexes were sedation and hypoxia since exposure, and decreased mean absolute spleen weights. At the mid dose, the NOAEL in both sexes, significantly decreased mean body and absolute organ weight changes were also observed. Thus, the low dose in males, 430 mg/kg/day, was considered the NOAEL. A 14-week neurotoxicity study, employing the same doses and exposure paradigm as the critical study, provided support for the sedation, hypoxia, and decreased body weight observed in the critical study. Since n-buty lactate is rapidly metabolized to n-butanal in the blood and brain, the likely mediator of the sedation and hypoxia caused by n-butyl acetate is n-butanal. Based on a total uncertainty factor of 3, 000x to account for inter- and intra-species, and the chronic extrapolation, as well as database deficiencies, the oral RD is calculated to be 0.1 mg/kg/day. This oral RD compares favorably to the IRIS oral RfD of 0.1 mg/kg-day for n-butanol. The resulting Short-Term Exposure Level (STEL) is 10 mg/L. Long-term exposure levels derived include a Total Allowable Concentration (TAC) of 1 mg/L and a Single Product Allowable Concentration (SPAC) of 0.1 mg/L. These drinking water action levels are considered to be adequately protective of human health.

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HUMAN RISK ASSESSMENT FOR γ-BUTYROLACTONE TO DETERMINE DRINKING WATER ACTION LEVELS


A risk assessment to determine acceptable levels of γ-butyrolactone in drinking water was conducted according to Annex A of American National Standards Institute/NSF International (ANSI/NSF) Standard 60/61, since it had been detected as a product extractant. Subchronic and chronic mouse and rat oral studies were evaluated. The key effect observed in these studies was general hypoxia, with progressively more severe neurotoxic effects observed as the doses increased. The effect was acute and occurred at a lower dose than any chronic effect. Further, the weight of evidence suggested that γ-butyrolactone was not genotoxic. γ-Butyrolactone is rapidly metabolized to γ-hydroxybutyrate. Therefore, neurotoxicity studies on this metabolite were also reviewed. From these, an acute human study in which twenty adult males were administered single oral doses ranging from 35-65 mg/kg γ-hydroxybutyrate was chosen as the critical study. The dose was administered at all dose levels, which were induced in subjects treated with doses of greater than 50 mg/kg γ-hydroxybutyrate. Electroencephalographic patterns similar to slow sleep were observed, in addition to alpha rhythms, in unconscious subjects. Brief delta and theta slowing was observed in subjects who were awake. This study was chosen because it was a human study and because the neurotoxicity induced by γ-hydroxybutyrate occurred lower than those in the repeated dose animal studies with γ-butyrolactone. This ensures that the resulting action levels would be based on the most sensitive toxicological endpoint. A LOAEL of 35 mg/kg was determined. Uncertainty factors of 10x each for intraspecies variability, database deficiencies, and the LOAEL to NOAEL extrapolation were used to calculate an oral RfD of 0.035 mg/kg-day. The Short-Term Exposure Level (STEL) was calculated to be 1 mg/L, and the Single Product Allowable Concentration (SPAC) were calculated to be 1 mg/L and 0.4 mg/L, respectively.

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IDENTIFICATION OF COMMON TOXIC EFFECTS WITH COMMON MECHANISMS OF TOXICITY FOR PESTICIDES SELECTED FROM THE DRINKING WATER CONTAMINANT LIST (CCL)


1Environmental Technology Division, Pacific Northwest National Laboratory, Richland, WA
2National Center of Environmental Assessment, USEPA, Richland, WA

Office of Pesticide Programs, USEPA, Washington, DC and Office of Water, USEPA, Washington, DC.

The Food Quality Protection Act (FQPA) has mandated that the EPA assess the cumulative risks to human health from aggregate dietary and non-dietary exposure to pesticides and other substances with a common mechanism of toxicity. It is necessary to identify chemicals or groups of chemicals that cause a common toxic effect based on a common mechanism of toxicity before assessing the cumulative exposure to chemical mixtures. In this study, the pesticides identified in the CCL FR 63(40), 1998, have been evaluated as candidates for cumulative risk assessment. A literature search was performed on MEDLINE, TOXLINE, and other databases using the CAS numbers for 40 candidate pesticides or major degradation products of known toxicological concerns. The extensive volume of retrieved literature, ~ 7000 citations with abstracts, was entered on to PNNL's Spatial Paradigm for Information Retrieval and Exploration (SPIRE) software to identify groups of compounds with a commonality of toxic effects and mechanisms of toxicity. The groups of compounds were later sorted based on specific toxic effects, i.e. hepatotoxicity, neurotoxicity, reprotoxicity, etc. The toxic effects groupings were viewed with SPIRE's visualization tools to identify pesticides or degradation products likely to possess common mechanisms of toxicity. Once relevant groups were identified, the selected scientific literature associated with each group was retrieved and the information used in the formation of a table of toxic effects and mechanisms of toxicity common to groups of compounds. This end product may be used as a basis for conducting cumulative risk assessment as needed.

DISCLAIMER: The opinions expressed in this study are those of the authors and do not necessarily reflect those of EPA.

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RISK COMPARISON OF SIX VOLATILE ORGANIC COMPOUNDS (VOCs) DETECTED IN CALIFORNIA DRINKING WATER


The California Department of Health Services maintains a drinking water monitoring database that consists of analytical data for more than 450 chemicals. In this paper, we evaluate the detection frequencies, average concentrations, and relative health risks of six VOCs in California drinking water that are classified as "known" or "potential" human carcinogens. Although not formally classified as a human carcinogen by the USEPA, methyl tertiary butyl ether (MTBE) is included in the analysis, given its recent controversy in California. We find that from 1995 to 2000, the percentage of drinking water sources containing at least one chemical detection is about 17% for chloroform, 9% for tetrachloroethylene (PCE), 8% for trichloroethylene (TCE), 2% for 1,1-dichloroethylene (1,1-DCE), 1.7% for MTBE, and <1% for benzene. Interestingly, the detection frequency for each VOC has not changed much over the last six years. Average detected concentrations from 1995-2000 are about 15 parts per billion (ppb) for PCE and TCE, 10 ppb for chloroform and MTBE, and 4 ppb for 1,1-DCE and benzene. Average detected concentrations have also remained relatively stable over the last few years, but exceed California’s Maximum Contaminant Levels (MCLs) for benzene, PCE, and TCE. To compare the relative risks posed by these VOCs in drinking water, estimates of lifetime cancer risks were also calculated for each chemical based on drinking water exposures via ingestion, inhalation, and dermal routes, and using average exposure parameter values and California-derived cancer slope factors. Estimated cancer risks are the greatest for 1,1-DCE, followed by PCE, benzene, chloroform, TCE, and MTBE. When adjusted for frequency of detections, PCE accounts for the greatest risk, followed by chloroform, 1,1-DCE, TCE, benzene, and MTBE. The results of this analysis provide useful information on relative risks of different drinking water contaminants in California, which can be used to prioritize public health hazards and aid in the development of appropriate risk mitigation efforts.
DERMAL EXPOSURE TO CONTAMINANTS WHILE SWIMMING: AN ASSESSMENT OF THE RISKS AND HAZARDS ASSOCIATED WITH USEPA AMBIENT WATER QUALITY CRITERIA.

H. Ochoa-Acuña, J. K. Tolson and S. M. Roberts, Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL.

Currently, Ambient Water Quality Criteria (AWQC) based on protection of human health consider intake from fish ingestion and, if the water is designated as a potable water source, from direct ingestion. Intake from dermal contact while swimming is not included in the development of these risk-based criteria. In order to evaluate the impact of this omission on the protectiveiveness of the AWQC, risks and hazards from dermal contact while swimming in waters with chemicals at AWQC concentrations were calculated. The assessment was focused on 42 carcinogens and 27 non-carcinogens for which AWQC are calculated based only on human health risks. For each chemical, Monte Carlo simulation was used to assess the variability of intake and risks among swimmers. Body weight data from NHANES 3 were used as a custom distribution, and surface area were calculated from allometric scaling based on body weight. A custom distribution for swimming frequency was taken from the 1994-95 National Survey on Recreation and the Environment. Swimming duration was entered as a triangular distribution with a minimum of 0 min, a maximum of 5 h, and a most likely value of 1 h. Dermal absorption was assessed using a non-steady state approach, given the limited duration of contact per each swimming event. For carcinogens, an exposure duration of 70 yr was assumed, and cancer slope factors were corrected for body weight. Among carcinogens, risks from dermal exposure for individuals at the 95th percentile ranged up to about 10^3, depending upon the chemical. For non-carcinogens, 95th percentile hazard quotients for dermal exposure ranged up to values well over 100. For some chemicals, virtually all swimmers in waters at the current AWQC would have an excess cancer risk > 10^-6 or a HQ > 1 from dermal exposure only. The results of this analysis suggest that dermal intake should be considered explicitly when developing surface water criteria for waters where recreational swimming occurs.

DETERMINATION OF A HUMAN ACUTE NO-OBSERVED-ADVERSE-EFFECT LEVEL (NOAEL) FOR COPPER IN BOTTLLED DRINKING WATER: VOLUME, DOSE AND CONCENTRATION CONSIDERATIONS.

K. A. Poirier, M. Araya, L. M. Klevay, F. H. Nielsen, J. Johnson, J. J. Strain, P. Robson, B. H. Chen, S. Wei, H. G. Zhu and S. R. Baker, TERA, Cincinnati, OH; University of Santiago, Santiago, Chile, USDA, Grand Forks, ND, University of Ulster, Coleraine, United Kingdom, Shanghai Medical University, Shanghai, China and TCA, New York, NY.

A human, acute NOAEL for nausea was determined in a prospective, double-blind controlled study. As part of an ongoing protocol, Phase II of this study recruited 70 adult females (18-60 years of age) at each of four different international sites (final pooled n = 269). The study design was a 3 x 3 factorial (volume x dose) for 100, 150, or 200 ml of bottled drinking water for 0.4, 0.8, 1.2 mg of Copper (Cu) as sulfate. Two additional doses (0 and 1.6 mg Cu) were added at the 200 ml volume to determine a Dose-Response and corroborate previously reported results. Each subject was given a blinded, randomly selected concentration of Cu once a week. Following dosing, all subjects were monitored by healthcare personnel for 1 hour. All subjects completed a questionnaire at 0, 0.25, 1, and 24 hours post-dosing that screened for positive GI effects (nausea, vomiting, abdominal pain, and diarrhea). Nausea was the most prevalent symptom reported and was generally reported within the first 15 minutes (water volume p < 0.032, copper dose p < 0.0601 and water x copper interaction p < 0.97). As volume increased, the effect of Cu-induced nausea decreased; as Cu dose increased; the incidence of nausea increased. At 200 ml, a significant increase in reported incidence of nausea at 0.25 hr occurred at 0.4 mg Cu/mL, indicating a NOAEL of 0.8 mg Cu (4 mg CuL) for adult females. An acute nausea threshold of 5.3 mg CuL can be calculated for the factorial analysis (0.8 mg Cu in 150 ml). These data confirm a previously determined human acute NOAEL for Cu and provide additional, controlled human data for determining regulatory contaminant levels of Cu for drinking water.

SCIENTIFIC CRITERIA FOR DERIVING OCCUPATIONAL EXPOSURE LIMITS FOR METALS AND OTHER COMPOUNDS RELATED TO MINING OPERATIONS.

A. Maier and L. T. Haber, TERA, Cincinnati, OH.

Despite general agreement that occupational exposure limits (OEL) play an important role in protection of worker health, significant disparities often exist in specific OEL values for a particular substance. These differences often reflect diversity in scientific criteria used among OEL-setting organizations. We investigated areas of scientific consensus and disagreement by conducting a critical evaluation of OEL documentation from various international organizations for a series of five case-study compounds: chromium, copper, lead, manganese, and silica. These chemicals served as a backdrop for evaluating key scientific criteria for setting OELs. As just one example, in the context of OELs for chromium compounds we present a decision logic for evaluating chemical speciation, demonstrate the impact of using particle dosimetry to calculate human equivalent concentrations from animal studies, and recommend approaches for considering nutritional essentially. Some issues became apparent only by comparing all of the case-study substances. For example, we found large differences in the extent of the supporting databases, but no explicit approach for documenting confidence in the resulting OELs. We also evaluated the application of uncertainty factors among various organizations and found that the rationale for their use, areas of uncertainty considered, and recommended defaults differed widely among organizations. Although in many cases we found that equally valid alternative approaches were used to address the specific issues we explored, the one over-riding theme in our analysis was the need to increase the degree to which the OEL documentation described the selected approach. The results suggest the opportunity for greater scientific harmonization in setting OELs, with the goal of increasing transparency in OEL derivation, and enhanced sharing of resources among organizations that set OELs. Sponsored by the International Council on Metals and the Environment.

DEVELOPING ENVIRONMENTAL CRITERIA BASED ON THE ACUTE TOXICITY OF LEAD.

B. K. Galgadhu, J. G. Pourdeh, T. W. Simon and S. M. Roberts, Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL, Pacific Northwest National Laboratory, Richland, WA and USEPA, Region 4, Atlanta, GA.

Effects from chronic lead exposure are a significant public health problem, and the development of environmental criteria for lead have understandably focused on circumstances of prolonged or repeated lead exposures. Occasionally, however, environmental problems arise where the potential for effects from acute lead exposure must be considered. Currently, no criteria are available for this type of exposure in environmental settings. In an effort to explore development of such criteria, the toxicological literature was evaluated with the objective of identifying blood lead concentrations that might serve as an upper limit to prevent acute lead poisoning. One approach to setting this limit would be to use the lower end of the range of blood lead concentrations observed in child and adult patients with symptomatic acute lead toxicity. A strength of this approach is that it deals with endpoints of obvious clinical relevance. An alternative approach would be to use the lower end of the range of lead concentrations associated with changes in hemolysis (e.g., 8-amino-creatinine dehydration, ALAD) as a sensitive endpoint. In basing the blood concentration limit on a sensitive biochemical effect of lead, this approach is likely to be protective for subtle acute effects of lead that might be missed in acutely poisoned patients. Blood concentration limits based on both approaches were compared to predictions of blood concentrations after various lead exposures using the Leggett model. The Leggett model was used because the IEUBK model is a poor predictor of blood lead concentrations resulting from acute exposure. With the Leggett model, upper limits on lead concentrations in environmental media could be calculated such that acute-exposed based blood lead concentration limits are not exceeded. This technique appears to hold promise as a method for developing environmental criteria based on acute lead toxicity.

EVALUATING LEAD EXPOSURE AT MINING SITES WITH HETEROGENEOUS SOIL TYPES AND LEAD BIOAVAILABILITY.

M. R. Garry and J. S. Tsiou 
Environmental Health, University of Washington, Seattle, WA and Depofens, Bellevue, WA

Risk assessments conducted on residential areas near mining and smelting sites typically apply USEPA's Integrated Exposure Uptake Biokinetic (IEUBK) model for children to evaluate lead exposure. The model is designed to estimate blood lead levels, using input assumptions about exposure to lead from various environmental media. Soil lead concentration and bioavailability are among the parameters with the most influence on blood lead exposures. At many mining sites soils are highly heterogeneous with regard to lead concentration, soil chemistry, and the associated soil lead bioavailability. This is due to both the natural mineralization of the area and the variable enrichment from mining activities. The IEUBK model is designed to accept only single input values, usually averages, for each parameter. In heterogeneous settings, use of independent averages for lead concentrations and bioavailability in soil inaccurately reflects lead exposure from either individual samples or from the site as a whole. This study describes application of a soil lead concentration-bioavailability weighted averaging method for deriving input parameters to the IEUBK model at a mining site in comparison with results derived using independent...
assessing bioavailability and other parameters influencing exposures to arsenic associated with cca-treated wood.


Potential health risks associated with arsenic originating in wood treated with chromated copper arsenate (CCA) are receiving increasing regulatory, scientific, and public attention. A recent risk assessment found that incidental ingestion of arsenic that may be present on the surface of treated wood structures (termed displaceable arsenic) was the primary contributor to exposure associated with arsenic from CCA-treated wood. Test evaluate this exposure pathway, several exposure parameters were derived that have not been frequently used in other risk assessment contexts, i.e., the concentration of displaceable arsenic on the wood surface (C\textsubscript{w}), the relative bioavailability adjustment (RBA) factor for ingested displaceable arsenic, and the hand transfer efficiency (HTE) factor describing the amount of arsenic that would be removed from the wood surface and subsequently ingested. Parameter estimates were derived based on data from the published scientific literature and other relevant studies. In addition, a sensitivity analysis was conducted to examine the variability and uncertainty of each parameter and the influence of parameter choices on risk estimates. Data sources reviewed in this work included studies of arsenic loadings on hands and feet materials, studies of arsenic bioavailability from CCA-treated wood and other solid media, information regarding the CCA wood treatment process, studies of soil adherence to hands, skin surface area data, and soil ingestion rate data. The results of the sensitivity analyses indicate that potential values for C\textsubscript{w} could span the widest range [with the 5th and 95th percentile values for wood samples differing by as much as a factor of 25]. By contrast, the high- and low-end estimates of HTE values differed by a factor of 14, while those for the RBA factor differed by only a factor of 2.3. These results suggest that actual arsenic exposures and risks are more likely to be less than the best estimates calculated in the risk assessment than to exceed them.

evaluation of human health risks from exposures to arsenic associated with CCA-treated wood.

E. M. Dube, C. G. Wells and B. D. Beck and S. Schetler. 'Gradant Corporation, Cambridge, MA and 'Gradant Corporation, Mercer Island, WA.

We performed a human health risk assessment (HHRA) to evaluate exposures to arsenic associated with chromated copper arsenate (CCA) treated wood. Central tendency exposure (CTE) and reasonable maximum exposure (RME) parameters were used to quantify exposures for a residential and a playground exposure scenario. The residential scenario included a male/female child ages 2-6 years, and a male/female child and adult ages 2-31 years. The playground scenario included a male/female child ages 2-6 and 7-12 years. Both exposure scenarios evaluated incidental ingestion and dermal contact with displaceable arsenic, which is arsenic on the surface of CCA-treated wood that can be removed by dermal contact with the hands. Eight different scenarios of treated wood were included in the evaluation. Exposure to arsenic in soil via incidental ingestion, dermal contact, and inhalation was also evaluated. The primary source of arsenic in soil was assumed to be a CCA-treated structure where CCA migrated from water runoff from the treated structure to the soil below. Estimated cancer and non-cancer health risks ranges from 9.9E-07 to 5.4E-06 and 2.6E-04 to 2.8E-02, respectively using CTE parameters, and 1.4E-06 to 1.2E-05 and 6.9E-04 to 6.4E-02, respectively using RME parameters, depending on wood sample and receptor. For all of the scenarios evaluated, 80 to 90% of the risks were from ingestion and dermal exposures to displaceable arsenic. The highest estimated risks were for a residential structure made of CCA-treated Southern Pine with pressure-applied water repellent. Estimated cancer and non-cancer health risks from the most commonly used type of treated wood in the US (i.e., CCA-treated Southern Pine), ranged from 1.1E-06 to 6.6E-06 and 6.9E-04 to 3.3E-02, respectively, for the resident. Based on the results of the HHRA, exposures to arsenic from CCA-treated wood in both a residential and playground setting does not pose a significant health risk to children or adults.

the use of a weight of evidence approach to assess health risks from arsenic exposure.


The importance realizing a weight of evidence approach when evaluating arsenic at contaminated sites became clear during two recent projects. Elevated levels of arsenic were encountered in two communities in Ontario, one near a former gold mine and the other near a sinning plant. Risk assessments, involving multi-pathway exposure assessment (air, water, soil, backyard produce, fish and market basket foods) and use of the USEPA slope factors revealed risk levels in the 1 to 10 range. Ontario considers 1 to 10, 000 to 0 to be the acceptable risk level. These elevated risk estimates raised community concerns. Answers were needed to ensure public safety and satisfy community and regulatory concerns. Further investigation into the risk assessment revealed the following: (i) market basket foods were the main contributor to arsenic related risks; (ii) generic criteria in Ontario (25 ppm) result in elevated risk levels (greater than 1 in 100, 000); (iii) the contribution of soil to overall arsenic related risks was small in all other pathways were less significant; (iv) health based intervention levels, as determined by the risk assessment, proved to be economically and technologically impossible; and, (v) removal of all soil above the generic criteria would result in a 2 to 4% overall risk reduction. It became clear that information beyond that typically contained within a risk assessment was needed to complete the decision making process. Therefore, limited health status studies were conducted which considered the incidence of cancer in the communities in question. In addition, urinary arsenic studies compared the 'impacted' communities with 'control' communities. In all cases, these studies indicated that the 'impacted' communities were similar to the control communities. Only after consideration of all pieces of evidence (risk assessment, community health status, urinary arsenic study) was it possible to conclude that there were no unsafe exposures or adverse health effects associated with elevated arsenic levels in both communities.

comparision of the toxicological profile determined in the Sprague-Dawley rat following dermal or dietary exposure to a prospective insect repellent active ingredient(KBR 3023).

B. S. Whale, S. G. Lake, L. P. Shreeve, G. K. Sangha and W. R. Christiansen. Toxicology, Bayer Corporation, Saltville, KS.

The general toxicological profile of -(1-methyl-propanoyl) (2,2-dihydroxyethyl) -piperidine (KBR 3023), a prospective new insect repellent active ingredient intended for human use, was studied in rats using the dermal and dietary route of exposure. Dermal applied dosages of 0, 80, 200, 500, or 1000 mg/kg/day were evaluated for 13 weeks (followed by a recovery period); dietary dosages of 0, 50, 100, or 200 mg/kg/day were evaluated for 1 and 2 years. Dietary dosages of 0, 100, 300, or 1,000 mg/kg/day were evaluated for 2, 5, and 14 weeks. With the exception of lesions occurring at the dermal dose site, the toxicological response of the rat following continuous and repeated dermal or dietary exposure to the test substance for durations of up to 14 weeks, was principally characterized by structural and/or functional alterations in liver and kidney-related endpoints. Lesions noted in the liver were indicative of an adaptive response to an increased need to metabolize and excrete an exogenous chemical. Lesions observed in the kidney were consistent with an alpha2 globulin-induced nephropathy; a common lesion in the male rat. By the end of the four-week recovery period that followed the 13-week dermal exposure, all changes attributable to exposure to the test substance had returned to within normal limits. Results following 1 and 2 years of continuous dermal exposure (excluding dose site lesions) were limited to an increased incidence of areas of cystic degeneration of the liver noted in males sacrificed at 2 years; indications of altered liver function were not observed. The same lesion was not identified histopathologically at 1 year. No evidence of a compound-induced nephritis was suggested. A comparison of the results of these investigations indicate a very similar systemic toxicological response to the rat following exposure to KBR 3023, regardless of the route of exposure.

differential species sensitivities and duration-specific reference concentrations (RfCs) for hydrogen sulfide (H2S) exposures.

T. Zowlel. Massachusetts Department of Environmental Protection (MADEP), Boston, MA.

H2S is an extremely hazardous gas and is the most common cause of death in the workplace. Although information on the human toxicity of H2S is abundant, exposure concentrations and durations are not well defined. Consequently, regulatory
agencies have had to use available animal data to derive RfC. A subchronic mouse inhalation study resulted in a LOAEL of 111 mg/m³ and a NOAEL of 42 mg/m³ for respiratory effects. Both MADEP and the USEPA derived a chronic RfC of 1 μg/m³ from the above mouse study using the NOAEL. A subchronic RfC of 10 μg/m³ can be developed using the same data. Because of the emergence of short-term human exposure data acute RfCs were developed and compared with the RfCs based on mouse data. Mild asthma symptoms exposed to 2.28 mg/m³ H₂S for 5 minutes subacute odor, and experienced nasal and pharyngeal dysynesia, bronchial obstruction and headache. This acute human LOAEL is 40 times lower than the mouse subchronic LOAEL.111 mg/m³ identified in the study that was used to derive the chronic RfC. A 30-minute acute RfC of 93 μg/m³ was developed by applying a safety factor of 30 (10 for the use of a LOAEL and 3 to correct for severe asthmatics) to the LOAEL. (2.78 mg/m³). In a separate study, field workers exposed to 125 μg/m³ H₂S for 5 hours showed persistent odor, and experienced eye and throat irritation, headache and nausea. If this LOAEL (125 μg/m³) is adjusted by a safety factor of 100 (100 for sensitive individuals, and 10 for LOAEL to NOAEL extrapolation), the 5-hour RfC will be 1.25 μg/m³, similar to the chronic RfC and lower than the chronic RfC. In conclusion, the emerging data suggest that (1) humans may be more sensitive than mice to H₂S toxicity; (2) the current chronic RfC may not be health protective and the interspecies extrapolation factor used to derive the chronic RfC should be re-evaluated for current toxicity data; and (3) future H₂S toxicity studies should be conducted in P₅₀ versus speciﬁc lethal, like rabbits and guinea pigs that are identified in the literature. (Abstract may not reﬂect MADEP policy).

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UTILIZING DATA FROM MULTIPLE STUDIES (META-ANALYSIS) TO DETERMINE EFFECTIVE DOSE LEVELS:

EXAM PLE OF MORTALITY IN RATS AND MICE EXPOSED TO HYDROGEN SULFIDE.


More chemical-specific risk assessments use a single best study to identify a toxicity marker, such as a NOAEL, benchmark dose, or LDE50. The objective of this exercise is to incorporate as much data as possible from multiple studies to derive a chemical-specific dose-duration response curve from which to identify toxicity markers. This approach has the advantage of incorporating more information than single-study assessments for a compound. For example, sensitivity of rats and mice, analyzed separately, from acute exposure to hydrogen sulfide. Methods of statistical analysis are used to determine when data from different studies can be pooled. EC₅₀, EC₉₀, and ESO₅₀ (concentrations at which there are response rates of 1%, 10%, and 50%) are estimated with 95% confidence intervals, at durations of 5, 10, and 30 minutes, and 1, 2, 4, and 6 hours. A concentration-response curve for mortality is fit to the rat data simultaneously for exposures of 5 minutes, 10 minutes, 30 minutes, and 1 hour, using a logistic curve additive in log(concentration) and log(duration). Separate curves are fit at 2, 4, and 6 hours. Fewer data are available for mice but fits are obtained for 30 minutes and 1 hour. The curves for rats fit the data exceedingly well and exhibit a threshold-like response followed by a steep incline as concentration increases. The response curves for mice start upward as lower concentration than for rats and then incline more gradually. Response is clearly more variable, perhaps due to different sensitivities of the species. The data on a mix of nonlethal adverse effects at the durations described above, and the observed NOAELs for mortality from individual studies, are plotted by concentration and described in the text. The NOAELs for mortality from individual studies fall within 95% confidence intervals, as described in the text. NOAELs for mortality from individual studies fall within 95% confidence intervals, as described in the text.

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THE ORAL REFERENCE DOSE FOR ETHYLENE DIBROMIDE: A NEW LOOK AT AN OLD PROBLEM.

M. Oster1, M. Odin, C. Smallwood2 and J. Swartzout2, 1Environmental Science Center, Syracoster Research Corporation, North Syracuse, NY and 2National Center for Environmental Health, USEPA, Cincinnati, OH.

Previous evaluations of the experimental toxicity data for ethylene dibromide (EDB) have concluded that limitations of the available studies precluded the derivation of oral toxicity values. Therefore, the USEPA has no oral RfD for EDB. At present, the USEPA has no oral RfD for EDB. Ultimate re-evaluation of the data, however, with consideration of the strengths and limitations of the existing studies, as well as a proposed mechanism of action for the toxic effects of EDB, a provisional oral RfD for EDB of 510 μg/kg-day is presented here. A lifetime carcinogenicity study in rats and mice was performed by the NCI, but was terminated prematurely because the MTD was apparently being exceeded. Even with early termination, dose-related increases in testicular atrophy were noted histologically, as well as increased incidence of tumors of the uterine vaginalis (the exterior covering of the tumor), Several studies in rats exposed orally to low doses of EDB also showed testicular atrophy, which was reversible upon cessation of exposure.

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NOAEL/LOAEL AND BENCHMARK DOSE APPROACHES TO SETTING ACCEPTABLE HUMAN EXPOSURE LEVELS TO 1, 2-DICHLOROETHANE (ETHYLENE DICHLORIDE).

D. A. Beblo1 and R. McGinnis2, 1Environmental Science Center, Syracoster Research Corporation, North Syracuse, NY and 2Environmental Science Center, Syracoster Research Corporation, Philadelphia, PA.

Chemical risk assessment approaches other than the most common NOAEL/LOAEL approach are being considered by the EPA. The benchmark dose (BMD) approach has a number of advantages over the traditional NOAEL/LOAEL approach, including not being limited to the experimental doses, and responding to sample size and the associated uncertainty. Because EPA's IRIS database contains no reference dose for chronic oral exposure (RfD) for 1, 2-dichloroethane, multiple approaches were compared for derivation of a provisional toxicity value. The majority of 1, 2-dichloroethane toxicity is consistent with reported injury to these organs in humans following accidental acute ingestion. The principal study selected as the basis for RfD derivation was conducted in rats and used five concentrations in drinking water, ranging from 500-8000 ppm, and a vehicle control. The lowest exposure level was considered a minimal LOAEL for increased kidney weight. Because the principal study examined a sufficient number of exposure levels, and RfD was also calculated using a BMD approach, RfD comparisons were based on several endpoints including increased kidney and liver weights, renal and liver histopathology, and altered enzyme activities.

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HEALTH RISK ASSESSMENT FOR TRICHLOROACETIC ACID.

D. Wong, Office of Water, OSTM/HEC, USEPA, Washington, DC.

TCA is one of the five haloacetic acids considered for regulation under EPA's proposed Stage 2 Disinfectants and Disinfection Byproducts Rule. A health risk assessment was conducted based on available studies. TCA induces systemic, noncancer effects in animals that can be grouped into three categories: alterations in intermediary carbohydrate metabolism, liver toxicity and developmental toxicity. The liver is the primary target organ for TCA toxicity. In a 104 week drinking water study in male F344 rats (DeAngel et al., 1997), there was a significant increase in body weight (10%), minimal histopathological changes in the liver, increased serum alanine aminotransferase level, and increased procoagulant proliferation at 364 mg/kg/day. The No-Observed-Adverse-Effects-Level (NOAEL) is 32.5 mg/kg/day. TCA increased resorptions, decreased implantations, and increased fetal cardiac malformations when administered to pregnant rats at 291 mg/kg/day on gestation days 1-22 (Johnson et al., 1998). In another study (Smith et al., 1989), decreased fetal weight and length, and increased cardiovascular malformations were observed when pregnant rats were administered 330 mg/kg/day TCA by gavage during gestation days 6 to 15. A RfD for TCA of 0.03 mg/kg/day is derived from the NOAEL of 32.5 mg/kg/day for liver histopathological changes identified by DeAngel et al. (1997). An overall uncertainty factor of 1000 is applied to the NOAEL to calculate the RfD. Mutagenicity data have been provided mixed results. TCA was reported to induce liver tumors in mice but not in rats. EPA has classified TCA as Group C: possible human carcinogen. Under EPA's Draft Guidelines for Carcinogen Risk Assessment, TCA can be described as having "Suggestive evidence of Carcinogenicity, but not Sufficient to Assess Human Carcinogenic Potential." (The opinions expressed in this abstract are those of the author and not necessarily those of EPA.)

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RE-EVALUATION OF THE ORAL REFERENCE DOSES FOR THE BROMINATED TRIHALOMETHANES BROMODICHLOROMETHANE, DIBROMOMETHANE, BROMOFROM.

L. H. Maullapras, B. C. Allen1 and N. H. Chung2, 1Environmental Science Center, Syracoster Research Corporation, Oxnard, CO, TPC Consulting, R.C. Crump Group, Chapel Hill, NC and 2Health and Ecological Criteria Division, Office of Science and Technology, USEPA, Washington, DC.

The brominated trihalomethanes (BTHMs) bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromofrom are by-products of drinking water chlorination. Information on the hazard, dose-response, and risk characterization
511 UPDATED ASSESSMENT OF HEALTH EFFECTS FROM TOLUENE EXPOSURE.


Toluene is widely used in industry as a solvent, gasoline additive, and in polymer production. The present EPA assessments for toluene on IRIS were verified between 1987 and 1990. Since the verification dates, a number of new studies have been published, warranting a reconsideration of the data for toluene. The existing IRIS RfD is based on hepatic effects in rats exposed by gavage. New oral studies not described on IRIS examined hepatic, neurological, and ophthalmological endpoints, and raise the issue of whether or not to change the critical effect for RfD derivation from hepatic effects to one based on immunological or neurological endpoints. The existing RfD is based on a LOAEL for behavioral effects in a study of toluene-exposed electronics workers, but a number of new studies examining neurologic effects in occupationally-exposed humans has been published, including two identifying NOAELs. The new studies support neurologic effects as the critical effect for RfD derivation and add more information about exposure-response relationships.

Several methods of analysis, including NOAEL/LOAEL and benchmark dose analyses, are under consideration with regard to the derivation of a new RfD. New data are also available examining the potential carcinogenic effects of toluene. While a single oral study in rats has suggested carcinogenic effects of toluene, limited reporting of the study results confounds interpretation. Adequate inhalation bioassays in rats and mice have not demonstrated a carcinogenic effect of toluene, and the vast majority of genotoxicity evaluations of toluene have been negative. (This document does not necessarily reflect EPA policy.)

512 REFINEMENT OF REFERENCE DOSE (RFD) FOR ZINC.

L. Ingerman, H. Chadbury, and M. Osiak. Environmental Science Center, Syracuse Research Corporation, North Syracuse, NY, and National Center for Environmental Assessment, Office of Research and Development, USEPA, Cincinnati, OH.

USEPA is currently re-evaluating the RFD for zinc. Derivation of an RFD for zinc requires balancing its essential functions with its potential adverse effects. Essential trace elements, such as zinc, pose an additional problem of nutrient-nutrient interactions. The excessive intake of one nutrient may interfere with the toxicokinetic properties of another nutrient. Increased zinc intake has been associated with a number of adverse health effects in humans, including decreased erythrocyte Cu-Zn superoxide dismutase (ESOD) activity, hematological effects, decreases in HDL-cholesterol levels, and gastrointestinal effects. Human studies provide evidence that excessive zinc intake may primarily induce copper deficiency. Excessive dietary zinc results in the induction of intestinal metallothionein synthesis. Since metallothionein has a greater binding capacity for copper than for zinc, copper absorbed into the intestinal mucosal cells is sequestered by metallothionein and does not enter the body. Copper deficiency is the primary mechanism for a number of the effects associated with excessive zinc intake, including decreases in HDL-cholesterol levels and ESOD activity. The endpoints associated with copper deficiency appear to be the most sensitive targets of toxicity (occur at doses of Zn as low as 300 ppm). There are limited data to suggest that high zinc intake can decrease iron absorption leading to symptoms of iron deficiency. In deriving an RFD for zinc, these interactions, as well as others, must be considered to determine a level of zinc (4-40 mg/kg) that would result in a dose below the no observed effect level. (This document does not necessarily reflect USEPA policy.)

513 RESIDENTIAL SOIL SAMPLING FOR CLEANUP DETERMINATION: COMPARISON OF FIXED-SITE LABORATORY AND FIELD PORTABLE X-RAY FLUORESCENCE (FPXRF) ANALYSIS AND SAMPLE PREPARATION.


Lead and other metals released from over 100 years of smelting and mining remain a public health concern in Idaho’s Silver Valley. In 1986, USEPA and Idaho Department of Environmental Quality started to remove contaminated soil from public areas located near the Bunker Hill Lead Smelter. Recently, USEPA has focused its efforts on sampling and cleanup of lead-contaminated soils at residences along the South Fork Coeur d’Alene River. Sampling objectives included analysis of soil for lead, arsenic, and zinc for cleanup determination as well as collecting data to test FPXRF as a predictor for fixed-site laboratory analyses (acid digestion/Inductively Coupled Plasma Spectroscopy, A/D/ICP) of dried and sieved samples (< 175 micron, mission-site fraction). FPXRF analysis was performed using a Niton XL 723. Results of FPXRF analyses of 1 dried and sieved samples; 2 dried samples; and 3 unprepared samples (at field moisture) were compared with dried and sieved samples analyzed by A/D/ICP. Linear regression models of the dependence of A/D/ICP on FPXRF yielded R-squared values (R²) that varied little among sample treatments, although the slopes of the zero intercept regression lines for lead were 0.92, 1.27, and 1.04 for groups 1-3, respectively. For arsenic, the R² values were: 0.97, 0.96, and 0.86. For zinc, the R² values were: 0.94, 0.86, and 0.94. These results suggest that, for these samples, predictions of A/D/ICP from FPXRF measurements would not have been highly affected by drying (moisture content) or sieving (particle size). Future sampling plans will use FPXRF to analyze unprepared samples with concerted A/D/ICP analysis of dried and sieved samples when FPXRF results approach soil cleanup levels. Benefits of FPXRF and reduced sample preparation include financial and logistical gains with potential to increase sampling density. Future plans include re-examining sampling and analysis protocols to apply real-time data acquisition to adaptive sampling techniques.

514 RISK ASSESSMENT OF METHYLMERCURY INTAKE FROM SPORT FISH TAKEN FROM SELECTED WATERBODIES IN THE NORTHERN SIERRA NEVADA FOOTHILLS, CALIFORNIA.

S. A. Klaing, R. K. Brodberg and A. M. Fink. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Oakland/Sacramento, CA. The United States Geological Survey collected samples of nine sport fish species from five reservoirs and 14 stream sites in the northern Sierra Nevada watersheds affected by historical gold mining. Total mercury concentrations were analyzed in composite and individual boneless and skinless fillet samples and reported on a wet weight basis. Mercury concentrations in collected fish species ranged from 0.02 to 1.53 ppm, with the highest concentrations found in spotted bass (mean = 0.85 ppm). In October 2000, the Departments of Environmental Health of Nevada, Placer and Yuba Counties, in cooperation with the Office of Environmental Health Hazard Assessment (OEHHAA), issued an interim advisory recommending that sport fishers limit their consumption of several species caught in the study area. OEHHAA has since conducted a more detailed assessment to determine human exposure to mercury and the associated potential health risks from consumption of these sport fish. Sample size was sufficient (SI) for exposure characterization at four species/fish combinations. Using the USEPA RFD for methylmercury (1x10⁻⁶ mg/kg/day), results of the assessment suggest that consumption by pregnant women and young children should be limited for sport bass from Camp Far West, largemouth bass from Lake Combie, smallmouth bass from Lake Englebright, and channel catfish from Rolison Reservoir. For other sites and species, data for similar species from the same water body or the same species from different water bodies were pooled to determine whether consumption should also be limited. Consumption restrictions are also recommended for all other bass species, channel catfish and bluegill from all evaluated reservoirs and brown trout and rainbow trout from the Bear River, South Yuba River, and Deer Creek sites. OEHHAA will issue final advisory for issuing sport fish advisories, will follow up in developing a final health advisory.

515 EVALUATION OF TEQ EXPOSURE FROM FISH CONSUMPTION RELATIVE TO AVERAGE POPULATION TOTAL EXPOSURE: IMPLICATIONS FOR PCB, PCDF AND PCDD RISK MANAGEMENT.

N. L. Judd, W. C. Griffith and E. M. Faustman. Environmental Health, University of Washington, Seattle, WA.

Recognition of a common mode of action for dioxins, dioxin-like PCBs and dibenzofurans has expanded our ability to assess cumulative risk from exposure to these ubiquitous compounds through consideration of TCDD toxic equivalency (TEQ).
PCBs are the second greatest cause of fish advisories, and PCBs are often the greatest contributors to TCDD-TEQ in fish. Because fish consumption is associated with both contaminant risks and health benefits, the incremental risk from this source should be considered within the context of overall TEQ associated risk. In this study, adult TEQ exposure from fish consumption using both historical fish consumption data and recent fish contaminant data from the literature is explored relative to average population exposure to these agents. Recent estimates presented by EPA suggest fish and seafood contribute just 19% of average diet exposure. However, results from our study indicate that for high-level consumers eating from relatively contaminated sites, TCDD-TEQ exposure from fish consumption alone may be 400% greater than the 1 pg TEQ/kg/day average estimated by EPA. This average exposure potentially carries an upper bound cancer risk of one in one thousand. Risk for other groups who consume high levels of fish and seafood from primarily commercial sources is likely less, but is far more uncertain, since data sufficiently sensitive to assess cancer risk from dioxin-like PCBs or even total PCBs is unavailable for over 96 percent of the seafood they consume. For most adult consumers, however, fish may contribute a small incremental risk from these compounds relative to an unacceptable ambient TCDD-TEQ associated risk, indicating the need for exposure reduction through more holistic environmental efforts rather than simple dietary changes. This proposal was prepared with support of USEPA Department of Energy, under Award No. DE-FG26-00NT40938. However, any opinions, findings, conclusions, or recommendations expressed are the author's and do not necessarily reflect the views of the DOE.

516  RISK ASSESSMENT OF POLYCHLORINATED DIPHENYLS (PCBs) IN INDOOR AIR.
B. K. Davis1, J. P. Beach2, M. J. Wade3, A. K. Klein4 and K. Hoch5. 1.Toxic Substances Control. California EPA. Sacramento, CA 2. California Tech. EM Inc. San Francisco, CA. In the 1970s, residences were built on a site where recent investigation found PCBs (principally Aroclor 1250) in soil at concentrations as high as 160, 000 mg/kg. Contamination was removed from a portion of the site, but concentrations under the residences were not removed or sampled. Though PCBs are relatively non-volatile, they are complex mixtures and there is a potential for transport of some constituents from soil into the foundation into indoor air. A screening analysis using USEPA's Johnson and Ettinger model for subsurface vapor intrusion estimated concentrations as low as 1000 mg/m³, corresponding to a cancer risk greater than 1-5·10⁻¹. The modeling was based on a saturation soil concentration of 168 mg/kg. Soil concentrations suggest the presence of non-aqueous phase PCBs in the soil, limiting the utility of the model. Five indoor air samples were collected in three sampling events over four months. Congener-specific analysis using GC/MS/MS measured total PCBs in indoor air ranging from 112 ng/m³ to 234 ng/m³. The SIM-MS detector has a low detection limit for risk management requirements, but may have resulted in artifacts of sampling, preparation, and analysis. Those potential artifacts required careful analysis of the analytical data to characterize air concentrations. The congeners measured in indoor air were mostly mono-chlorinated. Cancer risk estimates for the detected congeners range from 1·10⁻¹ to 5·10⁻¹. Conclusions: 1. The indoor air exposure pathway can be complete for low-volatility chemical mixtures in soil if they contain more volatile components and source concentrations are high. 2. Congener analysis provides important information to evaluate exposure to PCB vapors, because the congener distribution in air can be very different from that in soil. 3. The Johnson and Ettinger model is useful in identifying potential concerns. 4. Indoor air measurements are technically difficult, but can provide critical information about actual indoor exposure.

517  HUMAN EXPOSURE AND RISK ASSESSMENT OF METHIDATHION IN GREENHOUSES.
J. Byoun, K. Liu, F. Lee, H. Sung, K. Kim and J. Kim. School of Agricultural and Biological Science, Seoul National University, Suwon, South Korea 1. Division of Pesticide Safety, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon, South Korea 2. Korea Research Institute of Chemical Technology, Daedeog, South Korea 3. Korea Research Institute of Chemical Technology, Taejon, South Korea. Sponsor: M. Cho. Methidathion is a 3,5-dihydro-5-methoxy-2-oxo-1, 3, 4-thiadiazol-3-yethylmethyl-O,D,O-dimethyl phosphorothioate) is a non-systemic, organophosphorus insecticide registered for control of agricultural insect and mite pests on various terrestrial crops, such as cucumber, citrus and apple in Korea. Human health risk assessment of methidathion was evaluated in greenhouse situation for risk management and for re-entry risk establishment. Daily and incidental exposure to methidathion were measured when this chemical was mixed/loaded, applied, and during the harvest. And dermal penetration rate was estimated with in vitro dermal penetration test using diffusion cell. Oral ADI of methidathion is 0.002mg/kg/day and Oral NOAEL for human is 0.2mg/kg/day. The dermal penetration rate of methidathion was 13.92%/cm²/hr. The dermal exposure from mixing/loading or application was 2.69mg/hr and 115.6mg/hr, respectively. The average dermal exposure of harvesters for 2 days was found to be 1.08mg/hr. There was no significant evidence of inhalation exposure. Hazard Quotient (HQ) and Margin of Exposure (MOE) were used for short-term risk assessment of methidathion. There was no possibility of al-far mixing/loading, and harvest under the Personal Protective Equipment (PPE) situation.

518  ESTIMATES OF ACRYLAMIDE INTAKE FROM THE USE OF PERSONAL CARE PRODUCTS CONTAINING POLYACRYLAMIDE: A MONTE CARLO ANALYSIS.
G. A. Lawrence, C. Van Lennig and A. M. Shipp. IFC Consulting, Ruston, LA. Sponsor: M. Friedman. Estimates of the Lifetime Average Daily Dose (LADD) of acrylamide resulting from the use of representative personal use products containing polyacrylamide have been developed. Because each of the variables that are used to estimate intake can have a range of values and there is uncertainty as to which is the true value, a Monte Carlo analysis was conducted to make maximum use of all of the data. From these data, distributions of values were defined and used to provide a distribution of LADDs for each personal care product. Further, estimates of unit risk factors and doses corresponding to a target risk level. In this case, 1x10⁻⁵, were estimated from the bioassay data reported by Johnson et al. (1986) and Friedman et al. (1995) using those approaches typically used by the FDA, the USEPA, and proposed for use by the European Union (EU). Ratios of the estimated acrylamide intake at the median, mean and 90th percentile of the distribution of individual intake values to the lifetime dose corresponding to a target risk level of 1x10⁻⁵ were also calculated. All ratios were well below 1, indicating that all were below the target risk level. The conclusion can be drawn that the lifetime cancer risk from the use of polyacrylamide-containing personal care products, in the manner assumed in this Exposure Assessment, are well below acceptable levels. In all cases, even at the 90th percentile on the distribution of LADDs, the ratio of intake to the target dose ranged from 0.01 to 0.000002. Stated differently, use of some of these products and the resulting acrylamide intake that we would have to increase approximately 100 times at the high end of exposure by more than 500,000 times to reach the target level of 1x10⁻⁵. Even if it were assumed that an individual used all of the products together, the estimated intake would still provide a dose that was well below the target risk level.

519  ASSESSING POTENTIAL CANCER RISKS ASSOCIATED WITH INSECTICIDES USED TO CONTROL THE MOSQUITO-BORNE WEST NILE VIRUS: USE OF THE MARGIN OF EXPOSURE APPROACH.
M. B. Secker, S. Schetter, C. Liu, R. J. Blanchet and T. S. Bowers. Gradient Corporation, Cambridge, MA. The 1999 outbreak of the mosquito-borne West Nile Virus in New York City (NYC) resulted in the use of insecticides in an attempt to control mosquito populations. Because of the potential for human exposure to insecticides, a human health risk assessment was performed to describe and quantify the potential for health effects, including cancer, due to insecticide spraying. We evaluated cancer risks for six active ingredients in the insecticides, including the organophosphates malathion and naled, the pyrethroids permethrin, resmethrin, and sumithrin, as well as the synergist, piperonyl butoxide. With the exception of naled, there is some evidence that these active ingredients may cause cancer in humans, based on data from animal bioassays. Cancer risks are typically evaluated using a cancer slope factor (CSF). However, CSFs were only available for malathion and permethrin. Of the remaining potentially carcinogenic active ingredients, USEPA specifically recommends using a Margin of Exposure (MOE) approach for evaluating cancer risks for piperonyl butoxide, using the reference dose (RfD) as a point of departure (i.e., a dose associated with a negligible increase in excess cancer). In order to fully evaluate any potential cancer risks in the absence of a CSF or other recommendations, we also used the MOE approach for evaluating cancer risks due to sumithrin and resmethrin, using their RfDs as a point of departure. This poster discusses our rationale for using the MOE approach, and presents the results of our evaluation. For sumithrin and piperonyl butoxide, the MOE is sufficiently high (i.e., the exposure dose was sufficiently lower than the point of departure) as to indicate that cancer risks should be negligible. For resmethrin, the MOE indicates that although there may be some concern for increased cancer risk, excess risks are likely to be very low.

520  RISK ASSESSMENT OF A MILITARY PUBLIC GOLF COURSE SLATED FOR CLOSURE.
O. B. Hodoh1,2, M. A. Smith1,2 and T. W. Simon3. 1. Environmental Health Sciences, University of Georgia, Athens, GA, 2. Interdisciplinary Toxicology, University of Georgia, Athens, GA and 3. USEPA, Atlanta, GA. In 1993, Naval Air Station (NAS) Cecil Field was slated for closure by the Base Realignment and Closure Commission and approximately 17, 200 acres is planned for transfer to the private sector. Cecil Field is participating in the Installation
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BIODEGRADATION RULES OF THUMB: A MEANS TO REDUCE UNCERTAINTY ASSOCIATED WITH PREDICTING HALF-LIVES OF TOXIC, COMBUSTOR-EMITTED CHEMICALS DEPOSITED ONTO SOILS.

D. Knaebel, D. Gray, P. Howard, P. McCrimmon, E. Brady-Roberts and D. Williams, ESC, Synapse Research Corp., N. Syracuse, NY and USEPA, ORD/NCEA, Cincinnati, OH.

To address the risk presented by combuster-emitted chemicals deposited onto soils, this research examined if general trends, or "Rules of Thumb" could be derived that describe the basic influences that soil or chemical characteristics have on biodegradation half-lives. An exhaustive search of the scientific literature was conducted to find documents that provided half-life data and appropriate soil information. Data were extracted from these reports, entered into a database, and statistically analyzed. Soil characteristics examined were pH, organic matter content, soil type, and percent sand, silt and clay. Chemical characteristics examined were formula weight, melting point, water solubility, vapor pressure, Henry's Law constant, Octanol-water partition coefficient (K_{ow}), and soil partition coefficient (K_{oc}). The database provided summary information about the chemicals, the soils in which the chemicals half-lives were studied, and the range of half-lives observed. Statistical analyses permitted derivation of two Rules of Thumb, One Rule states that when a chemical is deposited onto a soil, its halflife will be longer in soils high in sand content compared to soils with less sand. The other Rule states that a chemical's half-life will be longer in soils with greater amounts of clay compared to soils with less clay. A predictive model was derived that allows estimation of half-lives for chemicals when they are deposited into different soils. The model has the form: Log_{10}(half-life) = 2.57×Log_{10}(FW) + 0.145×Log_{10}(salt) + 0.012×(silt + 0.753×Log_{10}(clay) - 8.018 (R^2 = 0.631, n = 110). Analysis showed, however, that more data are needed to apply this model to a wider range of chemical and soil types. In conclusion, the risk presented by combuster-emitted chemicals that are deposited onto a soil will depend in part on the sand and clay content of that soil. The Rules of Thumb capture the important elements of this relationship.

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EUROPEAN AND USA RBCA, COMPARISON OF HEALTH RISK ASSESSMENT RESULTS FOR OIL REFINERY SITES.

R. J. Welch, Toxscience, URS Corporation, New York, NY.

The potential for residential and industrial redevelopment of a large oil refinery sites were addressed using Tier 2 Risk-Based Corrective Action (RBAC) approach. Two software packages were used in the Tier 2 Risk assessment, (i) Risk Impact Evaluation on Hall and Institute, Netherlands, RISC-HUMAN, Version 3.1, 1999; and 2) Groundwater Services, Inc., USA, ASTM RBCA, Version 1.2, 1999. The RISC-HUMAN model is based on methodologies developed by the Netherlands National Institute of Public Health and Environmental Protection (known as RIVM). The GS1 model is designed to meet the requirements of the American Society for Testing and Materials (ASTM) PS-104 Standard Provisional Guidance for RBCA. There are several key differences in the models including: 1) the default exposure routes addressed; 2) the methodologies for evaluating carcinogenic risk; 3) the default human exposure and site parameters; and 4) the chemical fate and transport algorithms. Of particular note are the differences in the models for calculating the volatilization of contaminants from subsurface soil and groundwater to indoor air. A comparison is made between both the model methodologies and results of the risk assessment (i.e., back-calculated risk-based screening levels or RBLS).

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THE HEALTH SURVEY OF MUNICIPAL INCINERATOR WORKERS IN JAPAN.

Y. Ogawa, I. Morita, R. Yoshida, S. Yu, A. Nakara, R. S. Wang, S. Ueno, R. Bowler and N. Hisanaga, National Institute of Industrial Health, Kawasaki, Japan and San Francisco State University, San Francisco, CA.

It is well known that municipal solid waste incinerator (MSWI) fly ash contains heavy metals, dioxins, polychlorinated hydrocarbons, and other organic materials. The goal of this study was to assess the health effects of chronic exposure to fly ash MSWI workers. The subjects were 81 workers (mean age 42.7) from four MSWI's in the same city. Written informed consents were given from all subjects. Blood and urine samples were collected in the morning of each study day. Occupational health doctors interviewed each subject about his job history. examined the exposure to fly ash, and calculated its total duration. The subjects were classified into four groups: those were Long duration of exposure to fly ash, Short duration of exposure to fly ash, Limited exposure to fly ash, and Control. Blood chemistry, oxidative stress markers, immunological markers of sex related hormones, and neurobehavioral functions were measured and compared among the groups. There were no significant difference or association between measured variables and the groups except oxidative stress markers and neurobehavioral functions. The results of oxidative stress marker are presented elsewhere in this meeting (R. Yoshida). The 4-t score of POMS (Profile of Mood State), which is Tension-T tiredness, Depression-dejection, Fatigue-inertia, and Confusion-bewildenment, increased according to the duration of exposure. The study indicated a non-linear and significant relation between the measured variables and the total duration of exposure. Although we could not conclude that a certain contaminant of fly ash was responsible to these effects, there may be neuropsychological effects on workers exposed to fly ash.

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CHARACTERIZING THE HUMAN RISKS FROM MILITARY USE OF NON-LETAL WEAPONS (NLW).

J. Patterson, A. Mager and M. Doutonnier, TERA, Cincinnati, OH.

Non-Lethal Weapons (NLWs) are important assets in nontraditional military operations, such as peacekeeping missions, where the use of lethal force may not be a desired first response. Various types of NLWs are considered, including those employing chemical, electromagnetic, mechanical, or acoustic technologies. DoD directives call for these weapons to achieve an appropriate balance between the competing goals of having a low probability of causing death, permanent injury, and collateral material damage, and a high probability of having the desired anti-personnel or anti-material effect. A conceptual framework for characterizing the risks of human effects from non-lethal weapons has been developed. This framework focuses on the physiological effects caused by the weapons and utilizes tools and techniques for risk assessment, including Dose-Response and exposure information, and decision analysis to provide decision-makers with the probability of intended and unintended effects for the exposure scenarios of interest. Preliminary results indicate that force over distance is one unifying metric for some NLWs, and that Dose-Response curves for intended and unintended effects may be diagnostic for some NLWs, but not others. Field commanders and mission planners could use the resulting risk information to make informed choices with regard to which NLWs would provide the most appropriate combination of target effectiveness and risk for the particular situation or mission. This conceptual framework, and application of it to weapon systems based on new and current weapon systems, and new application of the traditional toxicology and epidemiology based risk assessment approaches. This work was funded by Department of Defense under subcontract PO F66050-DS0142.

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RECYCLING WASTES IN AGRICULTURE: EVALUATION OF THE QUÉBEC CRITERIA FOR CADMIUM AND DIOXIN/FURAN BASED ON HUMAN HEALTH ASSESSMENT.

M. O. Foucheceout and M. Beaudouel, Institut National de Sante Publique, Montreal, PQ, Canada, Sponsor: K. Kleinman.

In the province of Quebec, Canada, municipal or industrial wastes such as biosolids or calcined amendments can be used as fertilizer in agriculture if they respect several criteria including maximal concentration of 11 inorganic elements and of dioxins/furans (D/F). The objective of this study was (i) to evaluate the exposure of highly exposed individual (HEI) to cadmium (Cd) and D/F when biosolids and calcined amendments (CA) used as a main fertilizers have a concentration of Cd or of D/F equal to the maximal allowed, and (ii) to determine if the risk level is acceptable for the public health. Mean application rates (t d.w./ha) of poultry and pig biosolids (1.45 and 2.45 for primary biosolid (B1) and mixed biosolid (B2), respectively) and for CA (0.525) were determined from four scenarios of fertilization that
HAZARDOUS AIR POLLUTION: A RESEARCH STRATEGY.

M. J. Selgrade, NHEERL, USEPA, ORR, Research Triangle Park, NC.

The 1990 Clean Air Act Amendments list 188 chemicals/chemical classes as hazardous air pollutants (HAPs). EPA’s implementation of this act includes characterizing residual risks after applying technology-based standards for sources of HAPs, and developing an understanding of risks in urban areas from a subset of 33 HAPs, as well as risks from indoor and mobile sources. Nat’l Air Toxics Assessments (NATA) will monitor progress towards EPA’s goal to eliminate unacceptable risks of health problems from HAPs. The 1st NATA reveals many uncertainties. A research strategy is needed to reduce the uncertainties in risk assessment that result from lack of information. The research needed does not differ considerably from that needed to address the 6 criteria pollutants. The sheer number of chemicals is daunting. Thus, it is necessary to focus attention on those HAPs which pose the greatest risk to humans, and if possible group HAPs based on physicochemical properties, structures/activities, and mechanisms of toxicity, in order to increase the usefulness of information gathered on any one HAP. HAPs can pollute different media, hence, inhalation may not be the only or even primary exposure route. Research is therefore needed to consider the fate & transport of HAPs once they leave the source, to characterize personal exposure, and to consider methods for dealing with aggregate risks of exposure of multiple organ systems by more than one route. Because much of the current data come from studies using other than inhalation exposures, pharmacokinetic models are needed to facilitate route to route extrapolation. Information is also needed to deal with cumulative risk of exposures to HAP mixtures and to consider the risks of sensitive populations. Unlike the criteria pollutants, HAPs risk assessments will have to rely more heavily on animal to human extrapolation. The purpose of this symposium is to propose a multidisciplinary approach for meeting HAP research needs, provide some examples, and encourage the researchers to think outside the traditional box of one chemical, one route of exposure, one species studies. This abstract does not reflect EPA policy.

EXPOSURE ASSESSMENT TO HAZARDOUS AIR POLLUTANTS.

C. P. Weissel, EOHS/IECM, UMDNJ/RWJMS, Piscataway, NJ. Sponsor: M. Selgrade.

When Hazardous Air Pollutants (HAPs) are prioritized according to potential risk, it is critical to consider total exposure from both ambient and non-ambient emissions. Exposure to HAPs from non-ambient sources can exceed that from ambient emissions. Small sources of HAPs in close proximity to people (release within homes and use of personal products) can dominate the inhalation exposure and HAPs have other exposure routes (dermal/ingestion). For example, inhalation exposure to formaldehyde indoors with new furnishings, ingestion exposure to PAH from charcoal cooked foods, and ingestion and dermal exposure to dust containing lead and pesticides for toddlers can exceed inhalation exposures from ambient air sources. Characterization of HAPs by source type and physiochemical properties is important because not all HAPs can be individually determined in a cost effective manner. The contribution of ambient air emissions to indoor air is a function of environmental factors (concentration factors into homes). The indoor air concentration is critical to determine since people spend the majority of their time indoors or in transit. We have measured indoor/outdoor and personal/outdoor air ratios that were greatly in excess of one for many of the HAPs, such as the VOCs, chloroform, carbon monoxide and 1,4 dichlorobenzene and the aldehydes, formaldehyde.

HYDROCARBONS AND ENVIRONMENTAL CONSTRAINTS.

Cd and D/Fe concentrations were set to 0.5 and 10 and 24 mgCd/kg and to 17, 27-50 and 27 ng EQU/kg in Ba, B2 and Ca, respectively. The contamination of the soil resulting from these fertilizers annually was estimated for 100 years. The levels of Cd and D/Fe in food produced on this soil and on control soils (background concentrations) were estimated by the same method and compared. The HEI was defined as a farmer which spend all its life in a region where soils are mainly fertilised with biosolids and Ca, and which is exposed by ingestion (soil, biosolids, Ca, contaminated food), inhalation (vapours, dusts) and dermal contact. The method used to estimate the couse of exposure was validated by comparison of the doses estimated from the background concentration in soil with the ones published for Canadians by other authors. Following a long term (100 y), concomitant use of biosolids and Ca in the conditions described above, the dose of Cd and of the HEI increased by the amount of Cd in the biosolid (i.e., 0.84 mg/kg-d). The contamination by D/Fe would lead to a 65% increase in the risk of cancer related to the background level of risk, which is already higher than 1x10^6.

STRUCTURE-ACTIVITY APPROACHES AND DATA EXPLORATION TOOLS FOR PRIORITIZING AND ASSESSING THE TOXICITY OF HAZARDOUS AIR POLLUTANTS.


Hazardous Air Pollutants (HAPs) refers to a set of structurally diverse environmental chemicals, many with limited toxicity data, that have been grouped, not due to chemical or biological similarity, but rather due to a common route of exposure (inhalation), environmental media (air) and regulatory framework. Hence, this set of chemicals provides particular challenges to structure-activity relationship (SAR) and data mining approaches. These approaches can be productively applied to characterize and testing prioritization of HAPs chemicals, but analyses must reach beyond this set of chemicals to construct larger chemical groupings according to either common structures and mechanisms of action, or a common endpoint of concern. Commercial toxicity prediction programs can apply algorithms derived from large sets of chemicals to estimating general toxicity properties of the HAPs chemicals. Another type of SAR approach, more focused and exploratory in nature, has successfully characterized structural classifiers of activity and determinates of potency among a set of diverse volatile organics, many HAPs included, that have been evaluated for their ability to produce rat nasal histopathology. The electronic and reactivity indices identified have provided a basis for exploring associations with the sub-chronic nasal irritation endpoint for which larger data sets are available. Finally, a new initiative to enable structure-based searching and exploration of public toxicity data bases will be reported that: has the potential to greatly enhance current capabilities to gather relevant data with respect to HAPs chemicals. Such tools will facilitate analogue identification and hypothesis generation with respect to characterization of HAPs spanning multiple classes and toxicity endpoints of concern. This abstract does not reflect EPA policy.

CONCENTRATION - DURATION RELATIONSHIPS FOR NON-CANCER HEALTH EFFECTS OF EXPOSURE TO HAZARDOUS AIR POLLUTANTS.

W. K. Boyes, Neurotoxicology Division, NHEERL, USEPA, Research Triangle Park, NC.

EPA is charged with assessing the risks of both acute and chronic exposures to hazardous air pollutants (HAPs). The emissions from sources of HAPs are often characterized as temporally-averaged values, however, patterns of exposure not captured in such measures may influence non-cancer toxicity. This research program examined non-cancer health effects as a function of temporal exposure patterns, with the goal of improving models to predict toxicity across exposure scenarios. Currently, temporal adjustments in risk assessment typically are based on Haber’s rule (C x t = K) or (C x t = T), where C represents concentration, t time (duration) of exposure, K a constant toxic effect and n, when used, is empirically derived. Several general conclusions were derived from this work. Concentration is more important than time; where C and t were manipulated the influence of concentration predominated over time in contrast to the equal influence of C and t in the linear form of Haber’s rule. Target tissue dose. Understanding the relationship between exposure and target tissue dose was better at predicting toxicity than was Haber’s rule. Target tissue sensitivity changes over time: variations in target tissue sensitivity are not accounted for in standard C x t calculations. Interpretation of standard toxicity assays: standard toxicity assays do not address acute exposures during critical windows of sensitivity. Quantitative models: models with parameters reflecting physiological entities improved extrapolation across species and age as well as exposure concentration and duration. Understanding Mechanisms: having mechanistic information improved predictions across exposure conditions. These results demonstrate the importance of considering temporal exposure variations when predicting the risks of adverse non-cancer health outcomes. This abstract does not reflect EPA policy.
MODEL-BASED APPROACHES TO DOSE EXTRAPOLATION.

R. Sarangapani. The K. S. Crump Group, ICF Consulting, Research Triangle Park, NC.

The preponderance of non-inhalation route toxicological evaluations of volatile chemicals places a premium on methodologies for conducting route-to-route dose extrapolations. Such methodologies are increasingly important for industry and regulatory agencies seeking to conduct risk assessments of chemicals that pose a hazard predominantly by the inhalation route, because they facilitate the use of data from alternative dose-routes. For these compounds, a reliable capacity to perform route-to-route extrapolation increases the database for conducting risk assessments based on target tissue dosimetry. Physiologically-based pharmacokinetic (PBPK) models with route specific components link dose-routes through tissue dosimetry, and are the leading tool for conducting dose-route extrapolations. Model-based approaches to estimate dose-equivalence (in terms of mg per kg per time) for various exposure routes, such as inhalation, dermal, oral, stomach, and extraperitoneal have been computed for a few hazardous air pollutants (e.g. vinyl acetate and styrene). Minimum data needs to perform adequate route specific dosimetry, and their associated advantages and limitations, have been characterized. Such models can also be used for mode-of-action hypotheses testing by correlating multiple internal dose metrics to the observed response data.

AIR TOXICS RESEARCH NEEDS IN THE 21ST CENTURY: SUMMARY.

A. Hollan. Center for Environmental Health Sciences, University of Montana, Missoula, MT.

The 1990 Clean Air Amendments (CAA) listed 188 chemicals and chemical classes as hazardous air pollutants (HAP). In most cases the health effect of concern for HAP exposure is cancer risk, while a limited list of HAP's are known or suspected to cause or exacerbate other diseases (e.g., asthma, fibrosis, neurodegenerative diseases, developmental effects). The complexity of working with this extensive list of HAP's arises not only from the exhaustive list, but also the certainty that exposures will be to mixtures. This is further complicated by the limited information on exposure levels as well as health effects of individual compounds, nevertheless the effects of mixtures (additive, synergistic, antagonistic). Selection of representative classes of HAP's may be useful, but not necessarily resolve all of the issues and in the case of disease causation assumes that all of the potential mechanisms are identical. Furthermore, identification of sensitive and susceptible populations remains incomplete. The potential matrix becomes overwhelming and tactics need to be developed to focus on the most likely scenarios of exposure. One approach is to assess actual exposure that is most representative of a substantial fraction of the population and generate an appropriate mixture. Issues of review are the limitations of exposure assessment, the applicability of the resulting mixture, and the approaches that could be utilized having this information. Alternatively, in the absence of portions of this data set would be the most scientifically plausible approach to filling the data gaps. This information will be critical to advance the study of health effects to real world exposure to HAP's.

TOXICOLOGICAL APPLICATIONS OF METABONOMIC TECHNOLOGY.

D. G. Robertson. Drug Safety Evaluation, Pfizer Global Research & Development, Ann Arbor, MI.

Toxins, by definition, disrupt the normal composition and flux of endogenous biochemicals in, or through, key intermediary cellular metabolic pathways. These disruptions, either directly or indirectly, alter the blood that percolates through the target tissues. The diagnostic utility of any one trace biomolecule is limited by the number of variables affecting its concentration in situ and by the commonality of biochemical processes disrupted by toxins. However, if a significant number of trace molecules are monitored, the overall pattern, or “fingerprint” produced may be more consistent and predictive than any one marker. Obtaining such comprehensive biochemical information is made possible using high-field nuclear magnetic resonance (NMR) spectroscopy coupled with pattern recognition technology. Urine and serum are routinely studied, but virtually any biofluid can be used for metabolomic analysis. Magic-angle spinning NMR technology enables similar information to be garnered from tissues as well. The power of the technology lies in its ability to non-invasively assess animal physiology thus allowing temporal evaluation of the effects of metabolic flux alterations and toxicity. Onset and regression of toxicity can be determined within a single animal, target organs identified, mechanistic information obtained and biomarkers of toxicity established. When coupled with genomic and proteomic technologies, metabolomics permits complete assessment of toxicity from genotype through phenotype. The objectives of this symposium are to introduce the principles of metabolomics, describe its application emphasizing both the strengths and weaknesses and to present a vision of the future for this emerging technology.

METABONOMIC APPROACHES TO UNDERSTANDING THE CONSEQUENCES OF DRUG TOXICITY AND GENE FUNCTION.

J. K. Nicholson. Biological Chemistry, Faculty of Medicine, Imperial College, University of London, London, United Kingdom. Sponsor: D. Robertson.

High frequency 'H NMR spectroscopy provides a rapid method of characterizing and quantifying a wide range of metabolites in untreated biological fluids and tissues and a powerful means of exploring the biochemical effects of drug-induced toxicological processes. 'H NMR spectra of biofluids are highly complex, containing signals from thousands of metabolites representing many key biochemical pathways. The 'H NMR-generated biofluid metabolite profiles are characteristically changed in different toxicological conditions according to the exact site and mechanism of the lesion. Thus interrogation of the biofluid NMR data can give direct diagnostic information and aid the detection of novel biomarkers of toxic effect. Recent advances in MAS-NMR spectroscopy also allow high resolution 'H data to be collected on small intact tissue samples, thus giving metabolic information that is complementary to those obtained from biofluid NMR. By use of computer pattern recognition (PR) methods, complex biofluid/tissue NMR data can be reduced and analyzed quantitatively to provide PR maps showing the physiological and toxicological effects of test xenobiotics in pre-development drug toxicity studies and the effects of genetic modification. Neural network, rule induction and other expert system approaches can also be implemented to give direct diagnostic outputs on toxicity type based on NMR input data. NMR-based diagnostic techniques can be extremely sensitive for detecting the detection of low level damage in a variety of organ systems and is potentially a powerful new adjunct to conventional toxicological procedures for lead compound selection. With the advent of flow injection NMR technology, it is now possible to analyze hundreds of biofluid samples per day using one NMR spectrometer and to use NMR as a novel tool for toxicological screening. The NMR-PR and expert system approach to rapid in vivo toxicological assessment of drugs will be illustrated for a wide range of drug and toxin-induced lesions in both experimental animals and man.

APPLICATION OF METABONOMIC EXPERT SYSTEMS TO THE STUDY OF PHYSIOLOGICAL AND TOXICOLOGICAL VARIATION.


One of the major applications of NMR-based metabolomic technology in the pharmaceutical industry is the advanced chemometric analysis of NMR spectra of biological matrices with a view to establishing and predicting the pharmacological and pathophysiological consequences of drug candidates. Using such technology, overt toxic insults are easily detected as perturbations in the 1H NMR spectral profiles and expert systems can be constructed for efficient classification and prediction of drug toxicities. However, many factors can be responsible for generating biochemical alterations in the baseline urinary profile including change in diet, diurnal variation, hormonal cycles, changes in temperature, poor health, level of activity, genetic drift and bacterial contamination of urine, and all of these factors may have implications on the toxicologic response of an animal. NMR-based metabolomic methods can be used to establish the degree and precise nature of normal physiological variation for a population of animals or humans. Moreover, mathematical data filtering methods can be used to deconvolute confounding physiological effects, thereby removing sources of variation that are unrelated to the class of toxicity. Sophisticated expert systems that can compensate for physiological, pathophysiological and toxicological variation can thus be established.

METABONOMIC AND GENOMIC INVESTIGATION OF RENAL PAPILLARY NECROSIS (RPN).

C. E. Thomas1, T. P. Ryan2, H. Gao2, A. Moeckel2, J. M. Cole2, W. Dow2, A. Cawin1, C. Garaghan1, E. Holmes1 and E. Osterhede1. 1Toxicology Projects & Investigative Toxicology, Eli Lilly & Co., Mont-Saint-Guibert, Belgium, 2Investigative Toxicology, Eli Lilly & Co., Greenfield, IN and Imperial College, London, United Kingdom.

Metabolomics and oligonucleotide-based microarray technology were used in a collaborative study with compound 393277, an experimental agent inducing RPN. Bromoethanamine (BEA) was used as a positive control. Papillary injury was evident microscopically at 4 h postdose for 353277 and 24 h for BEA. NMR showed...
536 METABONOMIC TECHNOLOGY AND THE PHARMACEUTICAL INDUSTRY: THE COMET CONSORTIUM.

D. G. Robertson, Drug Safety Evaluation, Pfizer Global Research & Development, Ann Arbor, MI.

Metabonomics technology has the potential to impact drug-development from early in the discovery process through clinical trials. The non-invasive assessment of toxicity (or efficacy) enables rapid thorough screening while the potential for serial assessment of toxicity within a single animal minimizes drug requirements. Beyond screening, early identification of biomarkers of either efficacy or toxicity is engendered. This identification of biomarkers is more than just an alternative to traditional clinical chemistry measurements. Metabonomics enables identification of biomarkers where currently none exist. The non-invasive nature of the technique makes it especially suitable for use in the clinic. The dynamic nature of changes and the ease of identifying temporal responses allow for novel mechanistic information to be obtained on the impact of metabolism on health and disease. The pattern of value for the use of metabolomics techniques to toxicological endpoints is realized only if a valid pattern is established. The role of the consortium on metabolomics (COMET) is to establish a comprehensive database that defines the chemical space encompassed by hepatotoxins and nephrotoxins. The consortium is a research endeavor of 6 pharmaceutical companies collaborating under the auspices of Imperial College in London. This presentation will provide practical examples of the application of metabonomics technology within the pharmaceutical industry. "Lessons Learned" from the COMET consortium will be presented and cross-company validation efforts described.

537 TRANSPLACENTAL CARCINOGENESIS OF ENVIRONMENTAL TOXICANTS.

M. S. Miller1 and L. M. Anderson1. 1Weke Forest University, Winston-Salem, NC; and 2NCI, Frederick, MD.

A growing body of scientific evidence has shown that the developing fetus and neonate exhibit different sensitivities and responses to environmental chemicals than do adult organisms, and that exposure of parents before conception can alter the risk of cancer and other diseases in the offspring. Developmental and stage specific effects are often seen as a result of the dynamic nature of the developing organism, which presents a constantly changing biochemical picture as various enzymes and signal transduction systems are turned on or off during growth and maturation. The higher sensitivity of the fetus to certain carcinogens has long term implications for the regulation of potentially toxic chemicals. Several studies have suggested that the process of cancer induction may begin in utero following exposure of the fetus to carcinogenic agents. Thus, the fetus and neonate are not "little adults," and further understanding of the toxic effects of chemicals at both the biochemical and molecular/genetic level are needed in order to make rational regulatory decisions, to devise chemopreventive strategies for exposed populations, and to elucidate the unique characteristics of tumors resulting from damage initiated during the early developmental stages. The first speaker will discuss recent data showing that pre-conceptional exposure of male mice to carcinogens can lead to epigenetic changes in hormones that could influence tumor development in subsequent generations. The second speaker will review alterations that occur on oncogenic loci following in utero exposure of mice to 3-methylcholanthrene, contrasting the differences between adult and fetal exposure. The third speaker will continue on this theme and describe the role of the N-acetyltransferases in determining the genotoxicity of aromatic amines in the fetus and neonate. The last speaker will demonstrate the effects of tobacco smoke exposure on 4-amino-1-methyl-5-bromouracil adduct formation and mutation induction in the Hep-2 human epithelial and Raji lymphoblast cell lines to highlight the effects of in utero exposures on the fetus in the human population.

538 EPIDEMIC ASPECTS OF PERINATAL CARCINOGENESIS: HORMONAL MODULATION.

L. M. Anderson, Laboratory of Comparative Carcinogenesis, NCI, Frederick, MD.

Epidemiologic evidence continues to implicate early life exposures as factors in cancer risk, and certain forms of childhood cancer, including leukemia, appear to be increasing. Preconceptional exposures of fathers, occupationally to chemicals such as metals, solvents, and pesticides, and to tobacco smoke, have been linked to increased risk of childhood cancers. Preconceptional cancer risk after exposures of fathers has been clearly demonstrated in animal models, for many categories of chemical carcinogens and for radiation. The phenomenon in animals suggests that a novel mechanism may be in play, since the incidence of effect is higher by several orders of magnitude than the rate of any known specific locus mutation, and the inheritance pattern may be non-Mendelian. Since the tumors that are increased after preconceptional exposure of animals are most often those that have a significant spontaneous incidence, we postulated that the mechanism could be epigenetic and involve changes in hormones that influence tumor development. We have pursued this hypothesis using chromium(III) as the preconceptional exposure agent. Cr(III) is a common human occupational exposure metal and a demonstrated preconceptional carcinogen in mice. After preconceptional exposure to chromium, fetal offspring at 10 weeks of age have been found to have marked, highly significant alterations in average levels of serum corticosterone, glucose, and insulin-like growth factor 1, compared with offspring of vehicle-treated fathers. Since all of these factors have known potential to influence tumor growth, these findings confirm the hypothesis. To determine whether the hormone alterations relate in fact to tissue changes, we have utilized microarray analysis, starting with liver. The changes in corticosterone correlated strongly with gene expression for insulin-like growth factor binding protein 1, a regulatory protein for IGF1. Such transgenerational effects on hormones in offspring may have wide implications.

539 ALTERATIONS IN ONCOGENES AND TUMOR SUPPRESSOR GENES IN TRANSPLACENTALLY INDUCED TUMORS.

M. S. Milleg and M. C. Minoles, Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC.

Fetal mice exhibit differential sensitivity to carcinogens that is determined by their ability to metabolize xenobiotics. Following in utero exposure to 3-methylcholanthrene (MC), the treated offspring demonstrated increased sensitivity for tumor formation compared to exposed adult mice. [D2 x B6D2F1]F2 and Balb/c mice develop lung tumors 6-12 months after birth, with the F2 mice also developing liver tumors. This tumor incidence correlated with expression and inducibility of Cyp1a1. Conversely, fetal mice were resistant to tumor induction by 3-aminomethylindole (3-AMIM), 5-fluorouracil (5-FU), probably due to the lack of Cyp1a2 expression during gestation. MC-induced tumors from the [D2 x B6D2F1]F2 and Balb/c mice demonstrated a high incidence of mutations in Ki-67, with 79% and 56% of the tumors showing mutations. In [D2 x B6D2F1]F2 mice showed predominantly G→T transversions (84%) whereas the Balb/c mice had mostly G→C mutations (62%). In both studies, the type of Ki-67 mutation was associated with the histological stage of the tumors. Though no alterations were found in the p53 gene, alterations in the RB1/3544 gene were seen. All 14 of the larger lung tumors examined from [D2 x B6D2F1]F2 mice displayed decreased levels of Rb RNA and protein. A low incidence of alterations at the Ink4a locus in both lung (15%) and liver (27%) tumors was seen. However, whereas adult lung lesions exhibited mainly promoter hypermethylation and a low incidence of homozygous deletions, mice exposed in utero had low incidences of both point mutations and hypermethylation. The type of alteration at the Ki-67 locus appeared to influence the type of damage that occurred in Ink4a, suggesting that the type of mutation induced in Ki-67 may influence tumor progression. Thus, the developing organism is more sensitive to certain carcinogens and exhibits a distinctive tumor pathogenesis relative to adults.

540 PRE- and POSTNATAL EXPRESSION OF N-ACETYLTANSFERASES (NAT): POTENTIAL ROLE IN AROMATIC AMINE GENOTOXICITY.

C. A. McQueen, Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

Children and adults often respond differently to xenobiotics. This may be due, at least in part, to alterations in the expression of biotransformation enzymes that can affect the formation of reactive and/or non-toxic products. Studies in mice have fo-
cused on NAT2, an enzyme that may be involved in regulation of futile metabolism and play a role in neural tube development. NAT2 has been detected in marine pre-implantation embryos with the NAT2 protein and associated capacity for acetylation increasing from birth to senescence. Some activation of aromatic amines in cancer patients involves reactions catalyzed by NAT1 and NAT2. The development of expression of both genes is of interest. In C57Bl/6j mice, NAT1 and NAT2 mRNAs were detected at gestational day (GD) 10, the earliest time evaluated, with fetal N-acetylation of 4-aminobiphenyl (4ABP) measured at GD 10, 15, and 18. The ratio of N-acetylation of 4ABP was determined by quantitative RT-PCR, continued to increase with age. This trend was also observed when NAT activities were assessed. Hepatic N-acetylation of p-aminobenzoic acid, a murine NAT2 selective substrate, isozyme, a murine NAT1 selective substrate, as well as the carcinogenic aromatic amines 2-aminofluorene and 4ABP substrates for both isoforms, increased with increasing age. 4-aminexposure to 4ABP resulted in fetal 4ABP-DNA adducts, reflecting maternal and possibly fetal activation of 4ABP. Neuronal exposure to 4ABP resulted in levels of hepatic 4ABP-DNA adducts that were significantly lower at neonatal day 4 than in adult liver. This, the capacity to acetylate aromatic amine carcinogens is present pre- and postnatally. While fetal and maternal biotransformation contribute to 4ABP genotoxicity during gestation, there is sufficient neonatal biotransformation to result in 4ABP-DNA adducts. The activation of aromatic amine carcinogens and the formation of DNA adducts during development may result in critical mutations that increase risk of childhood or adult cancer. (Supported by ES 09812 and ES 10047).

541 APPLICATION AND USE OF HEMOCOLGIN AS A BIOMARKER OF TOBACCO SMOKE EXPOSURE.
S. R. Myers. Pharmacology and Toxicology, Center for Environmental and Occupational Health Sciences, University of Louisville School of Medicine, Louisville, KY.

Smoking during pregnancy has been linked to a variety of adverse outcomes, including low birthweight, spontaneous abortion, and infant death. Low birthweight shows the clearest and most consistent association with maternal smoking. Evidence suggests that a Dose-Response relationship exists between cigarette consumption, especially during the third trimester, and birth weight. Women who smoke during pregnancy expose themselves, as well as the fetus, to numerous carcinogens, many of which may be harmful to the fetus. Although much data concerning the effects of smoking is known, few studies have investigated the effect of tobacco smoke on the developing fetus. Three of the most widely studied carcinogens in tobacco smoke include the potent bladder carcinogen 4-aminobiphenyl, as well as the carcinogens benzo[a]pyrene and the tobacco specific nitrosamines. Numerous biological markers of exposure to tobacco smoke have been proposed and investigated. Recently, the utilization of hemoglobin (Hb) adducts as biomarkers of exposure to carcinogens has gained increased interest. Although Hb adducts are relatively non-specific, they have been utilized as biomarkers of exposure to various agents, such as those carcinogens found in tobacco smoke. In this presentation, we will discuss a series of studies that have helped define the use of Hb as a biological marker to assess exposure to carcinogens. In addition, we will discuss the following hypotheses related to the use of Hb adducts as biomarkers of exposure assessment. These include (1) that the formation of fetal Hb tobacco smoke carcinogen adducts is proportional to maternal smoking as well as maternal Hb adducts; (2) that the formation of maternal Hb adducts is dependent on maternal and fetal genotype to glutathione S-transferase M1 (GSTM1), glutathione S-transferase T1 (GSTT1), N-acetylationtransferases (NAT1 and NAT2), or cytochrome CYP1A1; and (3) that there is a correlation between the formation of DNA adducts and maternal and fetal Hb adducts.

542 INTRODUCTION TO PERFLUOROCARBOXISULFONATE (PFOS).

Perfluorooctane sulfonic acid (PFOS) is a perfluorinated organic acid that has been used as a surfactant in products such as fire fighting foams. It is also formed by the environmental or metabolic degradation of other fluorocarboxylic products used on items such as carpets, clothing, and food containers. PFOS does not appear to be further degraded by humans or environmental processes. Analytical techniques in the 1960s could identify organic fluorine in human serum at levels as low as 1.0 ppm. In the 1990s, improved methods and instrumentation allowed routine measurements of specific organofluorine chemicals in human and environmental samples at levels of 0.01 ppm. PFOS has been measured at low concentrations in serum samples from human populations, in various species of wildlife, in surface water, and in other environmental media. Occupational workers have measured serum PFOS levels that averaged 2.0 ppm with highest levels at 11 ppm. In non-occupational human samples, the upper 95th percentile serum concentration is approximately 0.1 ppm. The half-life of PFOS in the serum of retired workers is currently estimated to be several years. Environmental matrices are being examined to understand the dispersion of PFOS and other fluorocarboxylic in the food chain, general environmental and in the vicinity of manufacturing and heavy use. Results of such studies indicate that PFOS is widely dispersed in the environment. Substantial information has been developed and continues to be developed on the possible biological effects of exposures. The mammalian toxicology database has grown rapidly. Also, several epidemiological investigations examining workers have been completed. Numerous acute and chronic toxicity studies involving freshwater and marine organisms also have been conducted. The aggregate data, to be summarized at this workshop, can provide a basic understanding of exposure, effects, mechanism and risk to the environment, wildlife and humans.

543 UNDERSTANDING THE GLOBAL DISTRIBUTION AND ENVIRONMENTAL EFFECTS OF PFOS.

Perfluorooctane sulfonic acid (PFOS) and its salts are fully fluorinated organic molecules produced synthetically by electrochemical fluorination or from the degradation of other fluorocarboxylic products produced by electrochemical fluorination. As a fully fluorinated organic molecule, PFOS is very stable and resists further degradation. Substantial information related to human and environmental exposures to PFOS has been developed providing evidence of widespread distribution in humans and the environment. PFOS at very low levels has been detected in serum and tissue samples from both occupationally and non-occupationally exposed human populations, in various species of wildlife, and in surface waters and other environmental media. An extensive database has been built, and continues to be developed, on the possible biological effects of these exposures. Based on the existing body of information presented here, the observed levels of PFOS from a wide variety of samples have not been identified as adverse effects of human health, wildlife, or the environment. Additional data currently under development from ongoing studies will be used in the future to refine this initial assessment. This presentation presents a comprehensive ecological risk assessment of PFOS using existing exposure and effects data. Analytical methods for the determination of exposure concentrations and toxicology of PFOS are presented. The analyses suggest that levels found in the environment and in wildlife are not associated with adverse effects. Additional research is now underway that will be used to refine this initial assessment.

544 THE HEALTH EFFECTS OF PFOS.

Numerous repeat-dose oral toxicity studies on PFOS have been conducted in rats and primates. In rat studies, toxicity has been observed at doses of 2 mg/kg/day and above and effects include increases in liver enzymes, hepatic vacuolation and hepatocellular hypertrophy, gastrointestinal effects, weight loss, convulsions, and death. In rhesus monkey studies, toxicity has been observed at doses of 1.5 mg/kg/day and above, and effects include anorexia, emesis, diarrhea, hyperventilation, prostration, convulsions, atrophy of the salivary glands and the pancreas, marked decreases in serum cholesterol, and lipid depletion in the adrenals, and death. At doses as low as 0.75 mg/kg/day, changes in weight, growth, food consumption, changes in blood lipids, and decreased serum cholesterol and glucose, and weight gain have been observed. In a two-generation reproductive toxicity study in rats, all F1 pups in the 3.0- and 9.0-mg/kg/day dose group died within a day after birth while close to 30% of the F1 pups in the 1.6 mg/kg/day dose group died within 4 days after birth. The NOAEL and LOAEL for the F2 pups were 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on reductions in pup body weight. A subsequent cross-fostering study showed that the perinatal death was due to intrauterine death. In addition, reductions in serum lipids and cholesterol synthesis do not appear to play a significant role in the perinatal mortality. A report on the results of a two-year feeding study in Crl:CD (SD) rats is expected in late 2001. This abstract does not reflect EPA policy.

545 MOLECULAR MECHANISMS OF PERFLUOROCARBOXISULFONATE TOXICITY.
K. B. Wallace. Biochemistry & Molecular Biology, University of Minnesota, Duluth, MN.

The evidence regarding perfluorooctane sulfonate (PFOS) toxicity is strongly suggestive of a primary kidney disorder. Early indicators of exposure to PFOS in rats include kidney, hepatonephropy that results from expansion of the mitochondria, endoplasmic reticulum and peroxisomal bodies, decreased serum glucose,
triglyceride and cholesterol, and the accumulation of lipid vacuoles in liver tissue. In view of the fact that PCOS is a perfluorinated fatty acid, we questioned whether its toxicity might owe to competing reactions for fatty acid metabolism in the cell. Although PCOS and its metabolic precursor perfluorooctanesulfonamide (PFOSN) do not inhibit mitochondrial fatty acid oxidation, they do interfere with mitochondrial bioenergetics. PCOS causes a nonspecific increase in permeability of mitochondrial membranes, whereas the primary and secondary sulfonamides are highly specific and extremely potent uncouplers of oxidative phosphorylation. PFOS and its sulfonamide also interfere with fatty acid metabolism in the cell by activating peroxisome proliferator-activated receptor to stimulate the proliferation of hepatic peroxisomes and enhanced expression of peroxisomal enzyme activities in rats in tissue. Guinea pigs, however, are unresponsive to PCOS or PCOS-induced peroxisome proliferation. PFOS exposure also causes a dunamic stimulation of CYP4A1 expression in rats, but not guinea pigs. In conclusion, PFOS and its sulfonamide precursors exert multiple effects to interfere with intermediary metabolism by the cell, indicating that it is quite probable that metabolic dysfunction is the basis for the generalized wounding syndrome and perinatal mortality that characterize PFOS and PFOSN toxicity in rats. However, there exists a discerning species-related difference in susceptibility to metabolic dysfunction, which raises some uncertainty whether rats provide a reliable indicator of potential adverse outcomes associated with human exposures to PFOS or its precursor sulfonamides. (This work was supported by The 3M Company.)

546 CHALLENGES OF RISK CHARACTERIZATION FOR PERFLUOROOCTANESULFONATE (PFOS): A PERSISTENT, PERVERSIVE FLUOROCHEMICAL.

G. S. Omenn, University of Michigan, Ann Arbor, MI. Sponsor: J. Moore.

In traditional assessments used in risk characterization, external dose is generally measured or estimated in the absence of direct measurement of biomarkers of exposure in serum or other tissues. Absorption, distribution, metabolism, and excretion differences across species are often unknown; thus, generic uncertainty factors, not chemical-specific in nature, are commonly used. Such approaches can yield estimates of exposure and risk that are so widely divergent as to undermine the confidence of the scientific community. Scientific data for perfluorooctanesulfonate (PFOS) present characteristics that may permit more refined assessment approaches. PFOS has been identified in non-occupationally exposed human populations in serum samples at low parts per billion levels. The origin of PFOS in human sera is not clearly understood; most likely, it comes from a combination of environmental and product exposures. Because PFOS is persistent, serum levels integrate exposure over time from all sources. Furthermore, PFOS serum concentration has been shown to approximate liver tissue concentration in humans and in laboratory animals at levels observed in human populations. Using such knowledge can reduce some of the uncertainty inherent in the typical approach to risk assessment. The approach we recommend for PFOS involves comparisons of the range of serum levels documented in both epidemiological and toxicological studies of exposed populations. The presentation will examine these assumptions and discuss limitations related to the safety and extent of human exposure data, the assumption that PFOS is the sole or major toxicant, and the likelihood of genetic variation in biotransformation and in susceptibility to adverse effects.

547 EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) AND HYPOXIA ON CARDIAC VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION.

M. K. Walker, College of Pharmacy, University of New Mexico, Albuquerque, NM.

A myocardial oxygen gradient during embryo development is thought to stimulate coronary angiogenesis and VEGF is one factor that regulates this process. Previously, we have demonstrated that TCDD inhibits the branching of coronary arteries in the developing chick embryo heart. Thus, we tested the hypothesis that hypoxia can regulate cardiac VEGF mRNA expression and TCDD can reduce cardiac VEGF mRNA expression. To determine if hypoxic incubation conditions induced the cardiac hypoxia, chicken eggs were injected with 0.9% saline (control) or 4% miconazole (MISO, a tissue hypoxia marker) in saline on incubation days 7 or 9 (D7 or D9) and the incubated for 24 hr under 20% or 15% O2. Miconazole is reduced metabolically to a reactive intermediate when tissue oxygen levels are low and will bind to cellular macromolecules. Hearts were collected on D8 and D10 and analyzed for MISO binding by scintillation counting. Hypoxia induced a 15% ± 14% and 330% ± 60% increase in MISO binding on D8 and D10, respectively. To determine if these same hypoxic incubation conditions induced cardiac VEGF mRNA, chicken eggs on D7 and D9 were incubated for 24 hr under 20% or 15% O2. Hearts were collected on D8 and D10 and analyzed for VEGF mRNA by quantitative RT-PCR. Hypoxia induced a 169% ± 35% and 200% ± 31% increase in cardiac VEGF mRNA on D8 and D10, respectively. Then, in the effects of TCDD on cardiac VEGF expression at these same time points. Fertilized chicken eggs were injected with control or 0.24 pmol TCDD/g on day 0: hearts were collected on D8 and D10, and analyzed for VEGF mRNA by quantitative RT-PCR. TCDD significantly reduced VEGF mRNA on D8 by 25 ± 15%, but had no effect on D10. These results demonstrate that cardiac VEGF expression during coronary angio genesis is regulated, in part, by hypoxia and further suggest that TCDD can reduce VEGF mRNA during coronary development. Future studies will evaluate the ability of TCDD to inhibit hypoxia-induced VEGF mRNA on D8 and D10. Supported by NHM E059804.

548 TOXICITY OF CB 126 IN METAMORPHOSING SUMMER FLOUNDER LARVAE.

B. Soffientino¹, D. Nacci¹, and J. L. Specker¹. Graduate School of Oceanography, University of Rhode Island, Narragansett, RI and the URI, Atlantic Ecology Division, USEPA, Narragansett, RI. Sponsor: L. Mills.

The summer flounder, Paralichthys dentatus, is a commercially important species of flatfish found on the East Coast of the United States, from Cape Hatteras to Maine. Pelagic, stomachless larval metamorphosis into the benthic, gastric, juvenile flatfish in shallow coastal bays, lagoons and estuaries during the spring. In summer flounder, metamorphosis is regulated by thyroid hormones (TH), and chemically-induced hyperthyroidism inhibits metamorphosis past the very early stages. PDEs are major pollutants found in industrially impacted coastal ecosystems, and are known to act as endocrine disruptors by lowering blood TH levels in mussel and some fish. This project is concerned with determining the developmental effects of the dioxin-like CB 126 on metamorphosing larvae, and ultimately to understand if any of the effects are mediated by thyroid disruption. Here we present results of the first experiments carried out 30 day old (premetamorphic) and 39 day-old (early metamorphosing) larvae to establish their sensitivity to CB 126. Larvae were exposed to radiolabeled CB 126 through the water, and grown out for 3 weeks to assess mortality. Cytochrome P450 IA1 immunoreactivity was measured as a marker of Aryl hydrocarbon receptor (AhR) pathway activation in liver, small intestine, stomach, and kidney. Mean metamorphic stage was calculated at the end of growth to determine if sublethal doses had an effect on metamorphosis. The younger group appeared more sensitive than the older (LD 20 = 20.9 pg/mg and 170 pg/mg, respectively). Sensitivity to CB 126 was similar to that observed in embryos of other marine fish (e.g. Fundulus heteroclitus). CB 126 induction was highest in the kidney and liver, lowest in anterior intestine, and was maximal near the LD 20 in all tissues; this pattern was in accord with observations in other species. In the younger group we observed a dose-dependent trend toward more advanced metamorphic stage, suggestive of an effect of CB 126 on developmental rate.

549 EXPRESSION OF A TRUNCATED RETINOIC ACID RECEPTOR ALPHA IN PHENOTYPED INDUCED CLEFT PALATE.

J. Geléineu-va Waes, R. Barber, L. Starr, F. Alcoman and J. Bennett. Center for Human Molecular Genetics, University of Nebraska Medical Center, Omaha, NE.

The anticonvulsant drug phenytoin (PHT) has been widely studied with respect to its teratogenic potential to induce oralofacial clefts in the offspring of epileptic patients. The hypothesis that PHT-induced malformations involve perturbations in retinoid signaling pathways subsequent to altered gene expression, and, presumably, altered protein expression, was investigated in the current studies. Using in situ transcription/antisense RNA amplification techniques, and reverse Northern blots, our laboratory has previously shown that in utero exposure to PHT alters expression of the retinoic acid receptors (RARα, RARβ, and RARγ) in the developing embryos and palate. In the current study, alterations in expression of the RARα protein were examined immunohistochemically in in situ embryo sections following chronic in utero exposure to PHT. Using a commercial antibody to the C-terminal end of the RARα protein, and a custom antibody to the N-terminal end of the protein, we were able to demonstrate expression of a C-terminal truncated RARα protein in the cranial facial region of GD 14.5 PHT-exposed embryos presenting with a cleft palate phenotype. In control embryos, cells expressing the full length RARα protein in the region of palatal fusion were also expressing an epithelial-mesenchymal transformation. However, in PHT-exposed embryos, epithelial cells on the cephalic palatal shelves expressed the truncated RARα protein and did not undergo fusion, but rather, differentiated into columnar epithelial cells. RARα transgenic mice expressing alternate splice-site variants were quantified in a ribonuclease protection assay, and there was a significant increase in the alternatively spliced mRNA coding for the truncated RARα protein in the palates of PHT-exposed embryos. The relative increase in expression of a dominant-negative RARα protein following PHT exposure may affect transcriptional regulation of downstream growth factors necessary for normal craniofacial/palatal morphogenesis.
ROLE OF FOLATE IN FUMONISIN-INDUCED NEURAL TUBE DEFECTS.

J. Maddox, G. Bennett, L. Starr, F. Aleman and J. Gelineau-van Waez. Center for Human Molecular Genetics, University of Nebraska Medical Center, Omaha, NE.

The current studies investigate the mycotoxin fumonisin B1 (FB1) and its interaction with folate transport systems in the induction of neural tube defects (NTDs). FB1 is a contaminant of corn associated with a variety of diseases, and recent reports suggest it may act as a developmental toxin. Experimental evidence indicates that exposure to FB1 adversely affects folate uptake in vitro, potentially compromising cellular processes dependent on this vitamin. Since maternal folate deficiency has been causally linked to NTDs, we investigated this interaction as a potential mechanism for FB1-induced NTDs in a murine model. Preliminary results using inbred strains of mice indicate a differential genetic susceptibility to FB1-induced NTDs. SWV mice were completely resistant to the teratogenic effects of FB1, whereas there was a dose-dependent increase in the incidence of NTDs in the susceptible LMBc strain. Pregnant LMBc dams injected with 20mg/kg FB1 ip on GD 7.5 and GD 8.5 had a significant number of pups present with NTDs (81%). The precise timing of exposure during gestation was also critical, since a single FB1 dose on GD 8.5 resulted in only 16% cleft lip. Folic acid (50mg/kg) administered periconceptionally to pregnant dams was unable to provide protection and rescue the phenotype. FB1 inhibits the enzyme methylenetetrahydrofolate reductase, resulting in a decrease in biosynthesis of sphingolipids, important membrane components for GPI-anchoring of the folate receptor. Since depletion of cellular sphingolipids may disrupt folate receptor function, we examined FB1-induced alterations in folate transport by injecting pregnant dams with 1H-folate and measuring folate uptake in embryos. Our results indicated that 1H-folate uptake was significantly inhibited in FB1 exposed embryos compared to controls. Using 3H-labeled FB1, we were also able to demonstrate that FB1 crossed the placenta, inhibiting folate uptake within the developing embryo, and suggesting folate deficiency as a mechanism for FB1-induced developmental toxicity.

THE SENSITIZING POTENTIAL OF DIFFERENT FOOD PROTEINS IN AN ORAL BROWN NORWAY (BN) RAT FOOD ALLERGY MODEL.

L. M. Knipe, a, A. H. Pentinik, J. D. Astwood, b, E. Rice, c, G. Hellechtach, c, R. Thomas d and R. E. Goodman e. d Experimental Immunology, d TNO Nutrition and Food Research, e Ziwa, Netherlands and e Product Safety Center, Monteag, St. Louis, MO. Sponsor: f Van Blercken.

Brown Norway rats were sensitized with common strong and non-allergenic proteins to evaluate the potential predictive value of allergenicity for food proteins. Natural, purified peanut Ara h 1, shrimp tropomyosin, hen egg-white ovalbumin, potato patatin (the major allergenic protein in an uncommon allergen), and beef tropomyosin (a non-allergenic protein), were tested. BN rats (n=6) were either gavaged daily with 0.01, 0.1, or 1 mg protein/ml rat during 42 days, with or without the oral adjuvant: Cholera Toxin (CT), or sensitized by intraperitoneal injection (ip) with alum as a positive control. Weekly serum samples were tested for antigen specific IgE and IgG2a (a Thelper-2 mediated subclass in rats) antibody levels using a passive cutaneous anaphylaxis assay and enzyme-linked immunosorbent assay (ELISA). Although, responses to oral sensitization were not as common as to ip injection with alum, an apparent gradation of responses was observed in animals dosed with 1 mg per day without CT. Fifty percent of the animals developed specific IgG2a responses to the strong allergens Ara h 1 and Shrimp tropomyosin, 70% to ovalbumin, and one of six animals developed a specific IgE response to patatin. No animals produced IgG responses to beef tropomyosin. Interestingly Ara h 1, shrimp tropomyosin and ovalbumin are all stable in pepsin at low pH, a trait that is common for food allergens, while patatin is quite labile. Addition of CT as an oral adjuvant at day 0 and 7 during sensitization did not consistently increase the number of IgE responding animals. The reported results suggest that in the BN rat the oral route of sensitization, without the use of CT as an oral adjuvant, may be more predictive for food allergens than injection of the antigen with alum. In addition, based on the obtained results measurement of protein-specific IgG2a is not likely to be used as a stand-alone parameter for allergen sensitization.

ANTIBODY PRODUCTION INDUCED BY FOOD PROTEINS IN MICE: DOSE RESPONSES.

R. J. Deerman, a, H. Cadick, a, S. Stone, a, D. A. Baskett b and F. Kimble c.

a Research, b Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom and c Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom.

A limited range of produce, including peanuts, tree nuts and eggs, is responsible for the majority of IgE-mediated food allergy. We have examined the ability of food proteins of varying allergenic potential to provoke IgE and IgG antibody responses following systemic (intraperitoneal) ip exposure to BALB/c strain mice. Animals were treated with a purified peanut allergen (Arachis hypogea lectin) or with ovalbumin (OVA), a major allergic constituent of chicken egg. Mice were also exposed to proteins derived from the potato, a foodstuff for which there is little evidence of allergy. To the purified lectin (Solanium tuberosum), and to a crude potato protein extract (PPE), Animals (n=5 per group) received various concentrations of protein by ip injection on days 0 and 7. Seven days later, serum samples were analyzed for specific IgG content by enzyme-linked immunosorbent assay and for specific IgE content by homologous passive cutaneous anaphylaxis assay. Administration of OVA or peanut lectin provoked vigorous IgE antibody responses but relatively high titers at day 7 were elicited. Thus, titers ranged from 1/128 to 1/1,600 for 0.2% to 0.02% peanut lectin and from 1/62 to 1/106 for 10% to 1% OVA. Potato lectin (10% to 1%) stimulated similar titers of IgG antibody to those induced by administration of the same concentrations of OVA, but only induced low titer (positive with neat serum only) IgE at the maximum dose tested (10%). PPE was somewhat less immunogenic over the same dose range and also failed to stimulate marked IgE responses (titre of 1/2 at 10% only). Particularly taking into account the dose responses, these food proteins display a differential capacity to provoke IgE antibody which is consistent with their differing allergenic potential in man.

PEANUT PROTEIN ELICITS T HELPER 2 CYTOKINE PRODUCTION AS DETECTED BY GENE EXPRESSION AND PROTEIN SECRETION IN MICE.

B. F. Flanagan, a, J. C. Bets, a, H. Cadick, a, R. J. Deerman a and L. Kimble c.

a Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom and c University of Liverpool, Liverpool, United Kingdom.

Sensation to peanuts is associated usually with high titer IgE antibody, consistent with the selective activation of T helper (Th2) type 2 cells. We have characterized cytokine profiles, at the mRNA and protein levels, induced by exposure of mice to a purified peanut allergen, Arachnis hypogea lectin. Fourteen days following intradermal exposure of BALB/c mice to peanut lectin, draining lymph node cells (LNC) were isolated and cultured in the presence or absence of peanut lectin, con

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INTERLEUKIN-4 AND INTERLEUKIN-6 WERE INCREASED IN RATS SENSITIZED WITH TOLUENE DIISOCYANATE.

K. Zheng and M. Arima. Department of Preventive Medicine, University of the Ryukyu, Nishihara, Japan. Sponsor: W. Dong.

In order to investigate the cytokine profile in toluene diisocyanate (TDI)-induced occupational asthma, we conducted a quantification of cytokine production in a murine model of respiratory hyperreactivity to TDI. Wistar rats were sensitized with intranasal application of 10% TDI and provoked with 5% TDI to induce airway hyperresponsiveness. The blood leukocytes were counted, and bronchoalveolar lavage (BAL) was performed and the cellular responses in BAL fluid were analysed. Lung histological examination was performed to investigate the inflammatory status in the airway. The production of IL-2, IL-4, IL-6 and IFN-γ increased; in serum, BAL fluid and spleen cell were determined with ELISA kits. The cellular results demonstrated that neutrophils and eosinophils in blood were significantly increased and the total cells and each different cell, in particular eosinophils in BAL fluid were markedly increased in TDI sensitized rats. Histological analysis showed that a respiratory inflammation represented by prominent infiltration of eosinophils in central and peripheral airways was present in TDI-sensitized rats. The cytokine assays revealed that in TDI-sensitized rats, IL-4 was predominately secreted in serum, and IL-4 and IL-6, rather than IL-2 and IFN-γ, were predominately secreted in BAL fluid and in spleen cell. These findings suggested that IL-4 and IL-6 are preferentially produced and may have an important role in occupational asthma induced by TDI.

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SPECIES COMPARISONS AND STATISTICAL ANALYSES OF CYTOKINE FINGERPRINTING FOR CHEMICAL ALLERGENS.

R. Skinner, R. J. Dearman, E. V. Warbrick and I. Kimber. Research, Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom.

Prolonged topical exposure of BALB/c strain mice to different classes of chemical allergens induced cytokine secretion profiles consistent with the selective development of discrete functional subpopulations of T cells. Thus, treatment with the reference contact allergen 2,4-dinitrochlorobenzene (DNCB), which apparently lacks respiratory sensitizing potential, elicits a preferential type 1 cytokine production pattern, whereas the reference respiratory sensitizer trimellitic anhydride (TMA) stimulates a selective type 2 phenotype. We have now compared allergen-induced cytokine secretion profiles in the Brown Norway (BN) rat with those stimulated in the BALB/c strain mouse. Under conditions where TMA and DNCB induced similar levels of immune activation, divergent cytokine expression patterns are elicited. TMA-activated LNC isolated from both species elaborated high levels of the type 2 cytokines interleukin (IL)-10 and IL-13, but little of the type 1 cytokines IL-12 or interferon-γ. For vigorous IL-4 production, LNC from both BALB/c mice and BN rats required reactivation in vitro with the T cell mitogen concanavalin A. DNCB-stimulated LNC generally displayed the converse type 1 phenotype. Cytokine secretion profiles displayed by LNC derived from BN rats were considerably more variable than those observed for LNC from BALB/c strain mice. With the exception of IL-4, statistically significant differences (p<0.01) between TMA- and DNCB-activated LNC were recorded for all cytokines in BALB/c mice. For the BN rat, differences reached statistical significance (p<0.01) only for the expression of IL-13. These data demonstrated that the intrinsic ability of DNCB and TMA to promote preferential type 1 and type 2 responses, respectively, is species-independent. However, with respect to the identification of potential chemical respiratory allergens as a function of induced cytokine secretion profiles (cytokine fingerprinting) these data suggest that the use of the BALB/c strain mouse will provide the more robust method for hazard assessment.

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CHANGES IN THE COMPLEMENT SYSTEM IN TRIMELLITIC ANHYDRIDE-INDUCED OCCUPATIONAL ASTHMA IN THE MOUSE.

J.E. Regal and M. E. Mohrman. Department of Pharmacology, University of Minnesota, Duluth, MN.

Trimellitic anhydride (TMA) is a small molecular weight chemical known to cause occupational asthma. Recent studies in animal models suggest that the complement system plays a role in mediating symptoms of asthma. To determine if complement changes accompanying eosinophilia into the lung differ in TMA-induced asthma compared to ovalbumin (OA)-induced asthma, parallel murine asthma models were used. BALB/c mice were sensitized as follows: days 1 and 3 intradurally with either TMA or OA in corn oil vehicle; day 12 intratracheally with either TMA conjugated to mouse serum albumin (TMA-MSA), OA, or MSA as control. To elicit the allergic response, mice were challenged intratracheally on days 19, 22, and 23 with either 30 µg or 400 µg TMA-MSA, OA or control MSA. Eosinophil infiltration and concentrations of complement component 3 (C3) or the complement activation product C5a in bronchoalveolar lavage (BAL) were assessed 72 hr later in 4 to 15 animals per group. Mouse C3 was determined by ELISA and mouse C5a by a Western blot method. Challenge of a sensitized animal with 30 or 400 µg of the corresponding antigen resulted in significant eosinophil infiltration into the lung compared to MSA challenge. After challenge with 30 µg antigen, the concentration of C3 in the BAL increased in OA challenged mice, but not in TMA-MSA challenged mice. However, with 400 µg antigen challenge, the C3 in the BAL increased after either OA or TMA-MSA challenge when compared to MSA challenged controls. A significant increase in C5a in the BAL was only apparent after challenge with 400 µg TMA-MSA compared to MSA challenged animals, with no changes observed in OA challenged mice. These data demonstrate that different antigens elicit different changes in the complement system, and suggest that the mechanism of the asthmatic response may differ with the antigen. (Supported in part by NIH ES 07409.)

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PARA-PHENYLENEDIAMINE (PPD) REDUCES MONOCYTE CHEMOTACTIC PROTEIN-1 (MCP-1) LEVELS IN PPD/BB SPECIFIC T CELL CLONES, PBMCs, MONOCYTES, AND U937 CELLS.

T. Wahrle, D. Laihane, D. Kutz, H. E. Merk and B. Blomberg. Dermatology, University Hospital, Aachen, Germany.

Para-Phenylenediamine (PPD) is very strong allergen causing allergic contact dermatitis. We recently found that CD4 + T cells are involved in PPD contact allergy. PPD/BB specific T cell clones secreted high amounts of IL-4 and IL-5. However, little is known about the impact of chemokines in PPD allergy. Chemokines may play an important role in the development and excretion of delayed hypersensitivity reactions (Rollins, 1997). One CC chemokine, the monocyte chemotactic pro-
560  CYTOKINE PRODUCTION INDUCED BY LOW MOLECULAR WEIGHT CHEMICALS AS A FUNCTION OF THE STIMULATION INDEX IN THE LOCAL LYMPH NODE ASSESSMENT: AN APPROACH TO DISCRIMINATE CONTACT SENSITIZERS FROM RESPIRATORY SENSITIZERS.


In general, contact sensitizers have been shown to selectively induce Th1 immune responses, such as IFN-gamma production, whereas Th2 responses, such as IL-4 production, were seen after exposure to respiratory allergens. However, these features are to a large extent dependent on the dose of the particular allergen. Therefore, the aim of the present study was to investigate the distinction between sensitizers and respiratory allergens, by establishing dose-dependent cytokine profiles. The contact allergens 2, 4-dinitrochlorobenzene (DNCB) and benzene carboxymethyl cellulose (BCCMC) were used as positive controls for the experiment. IL-4 and IL-10 production was measured as a function of estimated sensitizations were calculated. All three respiratory allergens showed significantly higher IL-4 and IL-10 production patterns compared to DNCB and HCA. A positive identification of DNCB and HCA as contact allergens on the basis of IFN-gamma production, was observed only at very high stimulation indices (SI > 10). DNCB and HCA showed a lesser SI (SI > 10) for HCA. This approach combines potency evaluation and discrimination between contact and respiratory allergens in a single short assay. We propose that by direct linkage of proliferation and cytokine production in a dose-response manner, distinguishing contact allergens from respiratory allergens may be improved compared to present approaches.

561  AN APPROACH FOR SAFETY ASSESSMENT TO MINIMIZE POTENTIAL FOR TYPE I ALLERGY FROM PROTEINS IN PERSONAL CARE (PC) PRODUCTS.


This report summarizes an approach for supporting the safety assessment of proteins in PC products, e.g., hair care products, to minimize potential for inducing Type I allergies. There is increasing use of proteins (e.g., soy, wheat, plant extracts) in PC products. Currently there are over 3000 proteins, protein extracts listed in the NCI dictionary for commercial PC use. Proteins can induce IgE-mediated Type I hypersensitivity, that may lead to symptoms like urticaria and respiratory allergies. In general, use of proteins in formulations are usually low (<1% w/w). However, exposure to some proteins has caused allergy: urticaria, rash, hair products with quaternized hydrolyzed collagen (Ninomiya 1998; Allergy 53:107-87); urticaria rash PC products with hydrolyzed wheat (Vanrozen 2000, Allergy 55:924-96). A strategy for safety risk assessments of proteins is proposed using an example of a body lotion containing a protease. Aerosol exposures were measured during showering after dermal application of this product, that showed exposures of 8-11 ng/m3 of the protease. Dermal deposition studies also showed that 50% of applied protein remained intact on skin for over 24 hours. A previous report showed levels of 2-29 ng/m3 of a similar protein induced 5 of 61 patients after 6 months of daily shower exposure (Kelling et al., 1998. Allergy Clin. Immunol. 101:79-87). This proposed risk assessment approach depends on: (1) innate antigenic potency of the protein; (2) structural similarity to other proteins for SAR comparison; (3) exposure determination using relevant biologic route; (4) product application and usage habits; (5) comparisons to benchmarks. For the body lotion example used here it was concluded that this product was considered unsafe for consumers.

562  EFFECTS OF MANCOZEB ON IMMUNE SYSTEM IN AGRICULTURAL WORKERS.

C. L. Galli, F. Corati, G. De Pascale, S. Fusistioni, T. Mannione, S. Vienot, M. Maroni and C. Colessi. 1Pharmacological Sciences, University of Milan, Milan, Italy, 2Intern. Cnr. Pesticide Safety ICS, Busto Arsizio, Italy, 3Vigevano District. A.S.L. of Pavia, Pavia, Italy and 4Occupational Health, University of Milan, Milan, Italy.

Mancozeb (MBF) is a systemic fungicide that contains manganese and copper in a 2:1 ratio. It is known to cause respiratory allergy in agricultural workers exposed to this product. The aim of this study was to investigate the immune status of agricultural workers exposed to MBF fungicide (mancozeb). Twenty-six healthy subjects entered the study, 13 agricultural workers and 13 unexposed control subjects. The exposure to mancozeb was assessed through the determination of the levels of urine excretion of the main MBF metabolite, ethylendioxytetraethanolate (ETU). The study of the immune function was performed on serum and blood samples collected before and after the work shift in the workers and only once (morning sample) in controls. The parameters measured were complete and differential blood counts, serum immunoglobulins, complement fractions, autoantibodies, lymphocyte subpopulations. As for the functional area, we measured the proliferative response to several lymphocytes mitogens (PHA, PWM, anti-CD3 and PMA plus ionomycin) and cytokine production after stimulation (TNF-α, IFN-γ) in a whole blood assay. We found that agricultural workers resulted significantly exposed to mancozeb (median ETU level in urine: exposed= 9 mg/l; unexposed= 0.5 mg/l) then to mancozeb (median ETU level in urine: exposed= 9 mg/l; unexposed= 0.5 mg/l) then TNF-α, IFN-γ, IL-4, and IL-10 production were measured to assess the immune function. The results showed that the immune function was not affected by the exposure to mancozeb. However, the levels of TNF-α, IFN-γ, IL-4, and IL-10 were significantly higher in the exposed group compared to the unexposed group. The results of this study suggest that mancozeb exposure may affect the immune system, particularly the production of TNF-α, IFN-γ, IL-4, and IL-10. Further studies are needed to clarify the mechanisms underlying these effects and to understand the long-term impact of mancozeb exposure on the immune system.
can be used as a rapid and highly sensitive detector for toxicity evaluation and prediction. Cadmium (Cd) is an extremely toxic trace metal of concern to the US Air Force. Although toxic effects of Cd have been well characterized, its temporal effect on the transcription process has not been studied. To determine the transcriptional fingerprints of Cd toxicity, primary rat hepatocytes were exposed to Cd at 0, 1.25 and 2 μM for 2 hrs at 37 °C. Total RNA was isolated at -2 (pretreatment control), 0, 3, 6, 12 and 24 hrs post-exposure to determine mRNA expression profiles using the Affymetrix Rat 430A array. Simultaneously, hepatocyte viability was assessed by LDH leakage and MTT reduction. Our results indicated that (1) Cd resulted in a significant time-course-dependent transcriptional stress response (i.e., hem oxygenase, GCLC, MT1 and NQO1); (2) the NQO1 displayed a differential dose-dependent up-regulation due to Cd treatment: at 3 h-76 fold/5 fold; at 6 h-67 fold/8 fold (data are expressed relative to controls for the 2μM/1.25μM stress treatments); (3) 2 μM Cd down-regulated CYP450s, vimentin, and tyrosine aminotransferase at certain time points. Cd effects on gene expression profiles in rat primary hepatocytes were consistent with the results of transcriptomics data; however, transcript responses were more sensitive. In summary, Cd-exposed primary rat hepatocytes give rise to a unique gene expression fingerprint. Modulation of gene expression will help elucidate the mechanisms of Cd toxicity and identify early biomarkers that can be used to predict human health.

565 EXPRESSION OF A HEME OXYGENASE-LUCIFERASE (HO-LUC) CONSTRUCT IN TRANSGENIC MICE FOLLOWING TREATMENT WITH DIFFERENT CHEMICALS.


Heme oxygenase-1 (HO-1) is a key enzyme in the conversion of heme to biliverdin and may be involved in some cellular anti-oxidative responses. The HO gene has been previously shown to be rapidly up-regulated after treatment with chemicals or other inducers of oxidative stress. FVB mice transgenic for the luciferase gene under transcriptional control of the HO promoter (HO-luc) have been shown to express the HO-luc transgene similarly to the endogenous HO gene following the administration of HO-inducing compounds. In vivo tissue-specific expression of HO-luc can be visualized using a low-light imaging system (VIVIS) after i.p. injection of luciferin. The HO-luc mice are uniquely suitable for the previously shown to express luciferase in response to cadmium chloride, a potent HO inducer. In this study, compounds representing different toxicity pathways were used to evaluate the HO-luc Tg mouse as an in vivo screening tool for toxicity. Adult male and female HO-luc mice were treated (3-28 days) with acetaminophen (250 mg/kg, oral ip), cadmium chloride (CdCl2, 10 μM/kg, ip), chloroform (100, 200 and 250 mg/kg oral or ip), defibrate (500 mg/kg oral or ip), or dorosubrin (10 mg/kg intravenous). All compounds induced HO-luc expression with the greatest responses after CdCl2 or chloroform treatment, and tissue-specific pathology was confirmed. These findings suggest that the HO-luc mouse may be used as a model for in vivo screening of new and unknown compounds for their potential toxic effects.

566 UTILITY OF HISTORICAL DYNAMIC RANGE DATA IN MICROARRAY GENE EXPRESSION ANALYSIS.


A major difficulty in applying microarray gene expression (MGE) data to toxicity assessment is interpretation of the significance of a gene's induction or repression. One important component to the dimension of significance is the response of genes to different conditions, particularly in relation to their individual dynamic ranges of expression, which can differ significantly due to both biological factors and technical limitations within microarray platforms. In this study, we assess the utility of normalizing fold induction (FI) data based on individual gene dynamic ranges in the analysis and interpretation of MGE experiments with CdCl2, and other compounds reputed to exert pro-oxidant (OX) effects (chloroform, cyclosporin A, and purexamin monooxidase). Historical maximum (max) or minimum (min) FI values were derived from the Phase-1 database of in vivo rat MGE experiments, which includes 33 well-characterized, paradigm toxicants. FI data for each gene were then converted to % max or % min values by referencing corresponding historical values. In 6 hour liver samples from experiments with 3 rats treated with a single IP injection of 1 mg/kg CdCl2, the most highly induced (>2.5 FI in at least 2 rats) genes implicated in oxidative defense (OD) were heme oxygenase-1 (HO-1) (3, 3, 3, 4, 9) and metallothionein-1 (MT1) (5, 9, 21, 5, 12.5). However, when % max values were considered, the most highly induced (>40% of max in at least 2 rats) OD genes were Cu/Zn superoxide dismutase (Cu/Zn-SOD) (13%, 88%, 74%), heme-breaking protein 23 (HBP23) (23%, 63%, 83%), and cadmium-specific antioxidant (TSA) (6%, 45%, 82%). Induction values for both HO-1 and MT1 were >25% max for all 3 rats. Additionally, historical max data showed several instances of co-induction of Cu/Zn-SOD, HBP23, and TSA in liver by the other OD compounds.

567 MAP KINASE PATHWAY DECREASES THE INDUCTION OF METAL RESPONSE ELEMENT-DRIVEN LUCIFERASE ACTIVITY BY CADMIUM.

L. S. He, Y. Xia, D. W. Nebert and T. P. Dalton. Environmental Health, University of Cincinnati, Cincinnati, OH.

Both cadmium (Cd++) and zinc (Zn++) activate metallothionein (M1) gene transcription via the metal response element (MRE motif), to which the MRE transcription factor(s) (MRTFs) binds. In cells cultured in low Zn++, most MTFI is localized to the cytoplasm; however, even under these circumstances, a modest percentage of MTFI is in the nucleus and binds nucleic acid-dependent component. Activation of Mtr transcription by Zn++ is preceded by a stoichiometric activation of MTFI binding and its nuclear accumulation. On the other hand, Cd++ activates MTFI-dependent Mtr transcription without an increase in MRE binding or nuclear translocation. Thus, a modest amount of nuclear MTFI may acquire potent transactivation potential in the presence of Cd++. Because heavy metals appear to activate oxidative stress-signaling, we assessed the role of signaling components of mitogen-activated protein (MAP) kinase pathways in controlling the trans-activity of MRE. Transient transfection experiments were performed in monkey kidney fibroblast CV1 cells, using a reporter construct containing 153 bp of the M1 promoter fused to the luciferase (LUC) gene. Cd++ treatment resulted in a dose-dependent increase in LUC activity reaching a maximal 80-fold above control. Co-transfection with constitutively active MEK1, an upstream regulator of the MAPK kinase pathway, shifted the dose-response to Cd++ approximately 4-fold to the right and decreased to half the maximal induction. MEK1 and MKKS are the downstream mediators of MEK1. Co-transfection of both of these constitutively active forms similarly decreased the cadmium-mediated induction as MEKK1, PD98059, a general inhibitor of MRE, augmented Cd++ induction, either with or without MEK1 plasmid co-transfection. Using transient transfection with MTFI-null cells, these effects were found to be MTFI-dependent. Supported, in part, by NIH grants P30 ES06806 and RO1 ES04146.
chloride over a period of sixteen days, showed an increase in MT-1/2 protein, as well as an increase in MT-1E, -1X and -2A gene expression. MT-4 was induced in cells treated with 5 and 9 μM cadmium over the 16 day time course, whereas MT-3 mRNA was not detected in any of the chronic treatment results suggesting that MT-1 and -2 are induced by acute and chronic exposure to cadmium, whereas MT-3 is only induced by acute exposure to cadmium.

572 MICROARRAY ANALYSIS OF MOLECULAR CHANGES SPECIFIC TO CADMIUM-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELLS.

W. E. Achnavat, S. Quader, M. M. Webber and M. P. Wakelk

ACUTE CADMIUM EXPOSURE INDUCES STRESS-RELATED GENE EXPRESSION IN WILD-TYPE AND METALLOTHIONEIN-I/H NUL MICE.


ACUTE CADMIUM EXPOSURE INDUCES STRESS-RELATED GENE EXPRESSION IN WILD-TYPE AND METALLOTHIONEIN-I/H NUL MICE.


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ACUTE CADMIUM EXPOSURE INDUCES STRESS-RELATED GENE EXPRESSION IN WILD-TYPE AND METALLOTHIONEIN-I/H NUL MICE.
The purpose of this study was to investigate signal transduction pathways that participate in cadmium-induced regulation of metallothionein (MT), a thiol-rich metal binding protein with free radical scavenging properties. Using quantitative real-time RT-PCR, we show that in vitro cadmium (Cd) treatment of alveolar epithelial cells causes both time and concentration-dependent changes in MT1 levels of MTE1. The expression of MT1 was significantly elevated above control (25-fold) 4 hours after the addition of 10 μM CdCl₂. A maximum increase in MT gene expression (+ 100-fold) was achieved by 24 hours. Transcript levels remained above control at 15 hours (30-fold) and eventually returned to baseline levels by 24 hours. Exposure of cultures to 5, 10, or 20 μM CdCl₂, for 8 hours resulted in increases of MT expression of 25%, 100%, and 210-fold, respectively. Inhibitors, specific to protein kinase C (PKC), protein kinase A (PKA), ERK kinase (MEK), p38 mitogen-activated protein kinase (MAPK), and tyrosine kinases, were subsequently tested to determine how each affected Cd-mediated induction of MT-1. In these experiments, alveolar epithelial cell cultures were preincubated for 30 minutes with each inhibitor prior to an 8 hour treatment with 10 μM CdCl₂. Induction of MT by Cd was reduced by 70% when PKC activity was suppressed with the inhibitor GF19203X. In contrast, inhibition of MEK with PD90859 resulted in a 50% increase in Cd-mediated MT gene expression. Inhibitors of p38 MAPK and tyrosine kinase caused less dramatic changes in MT mRNA. In conclusion, the results of this study indicate that signal transduction pathways that include PKC and MEK as active components are of importance in the induction of MT-1 by Cd.

**ANTISENSE-BCL-2-INDUCED SELECTIVE OXIDATION OF PHOSPHATIDYLSERINE IN NCI-H226 CARCINOMA CELLS: ROLE IN APOPTOTIC SIGNALING.**


The major function attributed to Bcl-2 is its ability to confer resistance against apoptosis. We have previously shown that Bcl-2 is effective in preventing membrane phospholipid oxidation during apoptosis induced by a number of oxidants as well as non-oxidative stimuli. In particular, phosphatidylinerine (PS) was protected against apoptosis associated peroxidation and subsequent PS-dependent signaling events such as PS externalization. To further investigate the antioxidant/anti-apoptotic role played by Bcl-2 in oxidation of phosphatidylserine during cell apoptosis, we decreased the expression of Bcl-2 in the lung squamous carcinoma cell line NCI-H226 using a synthetic phospholipid oloigolipid targeted against bcl-2 mRNA (antinec-bcl-2). NCI-H226 cells (10⁶) were incubated with media only or with either the antisequence-bcl-2 (0.3 μM 5′-C1245′) oligolipid or with a specificity/cytotoxicity nonsense (0.3 μM 5′-C1245′) oligolipid control for 5 days at 37°C. Incubation of NCI-H226 cells in the presence of the antisequence-bcl-2 oligolipid resulted in a significantly increased amount of apoptotic cells as compared to controls. Using our technique to assess oxidative stress using cis-patric acid (PhA), we found a significant oxidation of PhA-A in apoptosis Bcl-2-apoptotic cells. No significant difference in oxidation of PhA-A labeled phosphatidylinositol and phosphatidylethanolamine between control cells and cells after treatment with both nonsense and antisequence-bcl-2 was found. This selective oxidation of PS cannot be attributed to changes of phospholipid composition as our HPTLC analysis of the major phospholipid classes showed no differences in the phospholipid composition between control (untreated) cells and cells incubated in the presence of either antisequence-bcl-2 and nonsense oligonucleotides. We conclude that Bcl-2 plays an important role in regulation of programmed cell death and PS oxidation during apoptosis.

**ROLE OF APOPTOSIS INDUCTION IN DEVELOPMENT OF LUNG INJURY FROM HIGH BURDENS OF TITANIUM DIOXIDE.**


We previously examined the role of apoptosis in lung pathology due to silica, a highly toxic particle and demonstrated that apoptotic cells and degradation products significantly accumulate as injury develops. In this study we test the hypothesis that titanium dioxide (TiO₂), a particle with known specific toxicity, induces apoptosis and accumulation of apoptotic degradation products. To test this, male, F344 rats were given intratracheal instillations (IT) of 0.4, 4 and 40 μg of TiO₂. At 1 day, 1 week and 4 weeks after instillation rats were sacrificed, lungs preserved by intra-tracheal instillation of fixative. Sections were processed for 1st labeling of apoptotic nuclei with a fluorescent indicator and counter-stained to label non-apoptotic nuclei. The number of apoptotic cell nuclei per lung was measured by morphometric methods. Additional sections were stained with Sirius Red to detect areas of collagen accumulation. Apoptotic cells in the saline instilled lungs were minimal at 1 and 4 weeks (0.2±0.2 and 0.3±0.4 million cells per lung, mean±SE). One week after IT, apoptotic cells per lung were 1.9±0.1, 4.4±0.4 and 13.3±2.2 million in the 0.4, 4 and 40 μg groups respectively. At 4 weeks the number of apoptotic cells was increased to 5.2±0.5, 9.5±2.2 and 24±3.1 million in the 0.4, 4 and 40 μg TiO₂ groups. Sirius Red staining of lung sections demonstrated areas with significant connective tissue accumulations in response to the IT of TiO₂ in both the 0.4 and 40 μg TiO₂ groups at 4 weeks of exposure. Sirius Red staining of collagen in saline and 0.4 μg TiO₂ groups was normal at 1 and 4 weeks. Our results demonstrate that high levels of inert, non-toxic dusts produce significant numbers of apoptotic cells and products. At high levels of lung burden the induction of apoptosis is associated with development of fibrotic foci. The results suggest that injury from the accumulated apoptotic products may be responsible for injury observed in high lung burden exposures to particulates such as TiO₂ which have no specific toxicity.

**BENZO(A)PYRENE 7, 8-DIHYDRODIOL IS GLUCORONIDATED BY HUMAN UGT1A9 AND CAUSES APOPTOSIS IN HEPG2 CELLS.**

S. Chen, M. Yurch, N. Nguyen and R. H. Tuley. Dept. of Pharmacology, UCSD, La Jolla, CA.

The human UDP-glucuronosyltransferases (UGTs) are involved in cellular detoxification and have been implicated as a mechanism to promote genotoxicity. Pyrocyclic aromatic hydrocarbons such as BENZO(A)PYRENE (BP) are metabolized through oxidation and epoxidation to simple and complex phenols. Both phenolic and dihydrodiol metabolites have been shown to be proximate mutagens capable of being formed and to form reactive electrophiles. Simple phenols are substrates for glucuronidation, little is known about the more complex dihydrodiols. Using expressed human UGTs (1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4 and 2B7), we have demonstrated that BP-7,8-dihydroidiol (±, 7,8-diol) is actively glucuronidated by UGT1A9. In cells deficient in UGT1A9, such as HepG2 cells, experiments were conducted to examine the mutagenic properties of 7,8-diol on cell viability. Annexin V analysis revealed in a dose dependent fashion that HepG2 cells were undergoing apoptosis following treatment with various concentrations of the 7,8-diol. DNA fragmentation assays confirmed the presence of internucleosomal cleavage products consistent with the analysis of poly (ADP-ribose) polymerase (PARP) as shown by the appearance of the PARP 89 kD fragment. In Western blot analysis, HepG2 cells treated with 2.5 μM 7,8-diol led to a two fold increase in cytosolic Cytochrome C, consistent with one of the properties of apoptosis. Bcl-2, an anti-apoptotic protein, decreased over 50%, while Bax, a pro-apoptotic protein, increased to 150% of that following vehicle treatment. In addition, an extensive down-regulation of xIAP (inhibitors of apoptosis) was detected which paralleled the appearance of apoptotic DNA fragments. In conclusion, HepG2 cells may be a sensitive biological tool to examine the actions of mutagens on apoptosis in addition to monitoring the genoprotective role of the human UGTs on cell viability. (Supported by USPHS grant CA79834)

**IN Volvement of oxidative stress in SDZ 1MM 125-Induced apoptosis in rat hepatocytes.**

A. Wolf, S. Grub and W. E. Trommer. Toxicology Pathology, Novartis Pharma AG, Basel, Switzerland and +Department of Chemistry, University, Kaiserslautern, Germany.

The mechanisms underlying the apoptotic activity of the immunomune suppressive drug cyclosporine A and its hepatotoxic O-hydroxethyl-D-ser/β-cyclosporine derivative SDZ IMM125 in rat hepatocytes are not fully understood. It was the purpose of the present study to investigate the role of oxidative stress in SDZ IMM125-induced apoptosis, and to evaluate the role of caspase-3 by coinubcation of anti- and pro-oxidants with rat hepatocytes. SDZ IMM 125 induced a dose-dependent increase in chromatin condensation and fragmentation, and the activation of caspase-3. Supplemeting the cell cultures with the antioxidants, DL-α-tocopherol polyethylene glycol-1000-succinate and ascorbic acid significantly inhibited the SDZ IMM 125-mediated increase in chromatin condensation and fragmentation, and caspase-3 activity. The reducing agent, dihydrothreitol, significantly inhibited the SDZ IMM 125-mediated increase in chromatin condensation and caspase-3 activity. The glutathione synthetase inhibitor, buthionine sulfoximine, significantly enhanced SDZ IMM 125-mediated caspase-3 activation and increased SDZ IMM 125-mediated chromatin condensation and fragmentation. The present data suggest that SDZ IMM125-induced apoptosis is mediated by the imbalance of anti- and pro-oxidant steady state in rat hepatocytes and that the intracellular redox-state can act as a modulator of apoptosis. The results further suggest that antioxidants and pro-oxidants might act on apoptosis by modifying caspase-3 activity.
579 REVERSAL OF ACETAMINOPHEN (AAP) AND THIOACETAMIDE (TAM)-INDUCED CASPASE-ACTIVATED DNASE (CAD) AND OXIDATIVE STRESS-MEDIATED APOPTOTIC AND NECROTIC LIVER CELL DEATHS BY MOROMIDICA CHARANTIA BIOFLAVONOIDS.

S. D. Ray, T. S. Lam, A. Dontabakhtuni, J. A. Roollo, S. Phadke, R. R. Raje and D. Bagchi, Molecular Toxicology Program, Division of Pharmacology & Toxicology, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Brooklyn, NY.

Acetaminophen and Thioacetamide are well known for their liver-specific apoptogenicity. Similarly, bitter gourd or M. charantia extract (MCE), has been vouched nearly a century, for its anti-diabetic, anti-inflammatory and hypolipidemic properties. However, the influence of MCE on apoptosis-inducing signals in vivo remains unknown. This study investigated effects of MCE pre-exposure on AAP- and TAM-induced hepatotoxicity, and events that orchestrate apoptosis (oxidative stress and CAD-dependent genomic DNA fragmentation). Additional focus was to determine the incidence of apoptotic and necrotic deaths and the degree of cytotoxicity. Male ICR mice were given orally MCE for 7 and 14 days. On 7th or 14th day, AAP (400 or 500 mg/kg) or TAM (25, 50, 100 mg/kg) was administered ip. All animals were sacrificed 24 hrs after AAP or TAM injections. Blood samples were collected to analyze serum chemistry, and liver tissues for biochemistry and histopathology. Results show that MCE pre-exposure by injection or feeding, significantly counteracted by MCE pre-exposure. In addition, apoptosis controlling events, such as oxygen DNA fragmentation and oxidative stress (MDA accumulation and glutathione depletion) culminated by AAP and TAM were also significantly inhibited. Histopathological evaluation of liver sections by brightfield microscopy (X1000x) revealed, both AAP at TAM caused dramatic necrotic cell death. Interestingly, MCE-AAP exposed tissues displayed near complete reversal of AAP-induced effects, whereas MCE-TAM-treated sections exhibited significant protection from TAM-induced hepatotoxicity. These findings suggest that, besides chemopreventive properties, MCE may influence drug and chemically-induced apoptogenic and necrotic signaling mechanisms in the liver.

580 MOLECULAR MECHANISMS OF ZINC INHIBITION OF ETHANOL-INDUCED LIVER APOPTOSIS.

J. C. Laneher, Z. Zhou, and Y. L. Kang, 1Pharmacology and Toxicology, University of Louisville, Louisville, KY and 2Medicine, University of Louisville, Louisville, KY.

Apoptosis is critically involved in the hepatic pathogenesis induced by acute alcohol exposure. This study was undertaken to test the hypothesis that zinc interferes with apoptotic signaling pathways in the liver leading to inhibition of ethanol-induced apoptosis. Male 129/SvJ mice were injected daily with 15% ethanol (5 g/kg) in 12 h intervals for 24 h, prior to intragastric administration of ethanol (5 g/kg) in 12 h intervals for 36 h, which has been shown to represent binge drinking in humans. Ethanol-induced apoptosis in the liver was detected by a terminal deoxynucleotidyl transferase nick-end labeling assay and further confirmed by electron microscopy. The number of apoptotic cells and Western blotting analysis. Immunohistochemical analysis revealed that zinc inhibited ethanol-induced endogenous hepatocyte apoptosis activation, which is a key component in signaling pathways leading to hepatic caspase-3 activation and apoptosis. Further studies showed that zinc suppressed ethanol-induced caspase activity, as determined by enzyme assay and Western blotting analysis. These results thus demonstrate that zinc is a potent inhibitor of ethanol-induced liver apoptosis and that this inhibition occurs primarily through zinc interference with Fas/Fas ligand signaling pathway.

581 DIFFERENTIAL INFLUENCE OF STREPTOZOTOCIN (STZ)-INDUCED HEPATIC CYTOKINES ON ACETAMINOPHEN (AAP)-MEDIATED APOPTOTIC CELL DEATH AND EXPRESSION OF CYTOKINES IN THE LIVER.

L.A. Roslo and S. D. Ray, Molecular Toxicology Program, Division of Pharmacology & Toxicology, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Brooklyn, NY.

The diabetic condition and toxic doses of AAP are known to increase CYP2E1 levels, oxidative stress, and promote apoptotic cell death in the liver in addition to modulating cytokine levels. This study was specifically designed to investigate whether diabetic hyperglycemic state, potentiates or antagonizes AAP-induced: i) hepatotoxicity and genotoxic DNA fragmentation, ii) oxidative stress, iii) cytokine expression, and iv) apoptotic cell death. Hyperglycemia and liver injury were induced by ip injections of STZ (50 mg/kg) and TAM (400 and 400 mg/kg for 24 hrs) respectively. Male ICR mice were administered Vehicle, STZ, AAP, or STZ-AAP. Blood was collected to evaluate glucose levels and serum chemistry, and livers for histopathology, analysis of DNA integrity, and cytokine levels. Surprisingly, results indicate that STZ-induced hyperglycemia potentiated liver DNA fragmentation, but antagonized hepatic cytokinopathy and oxidative stress. Serum ALT activities were 3.4 and 7.8 fold lower in STZ-AAP groups compared to AAP alone controls. Oxidative stress markers, lipid peroxidation and total glutathione contents, mirrored the serum chemistry changes. In contrast, the diabetic condition increased DNA fragmentation (Con: 100%; STZ: 206%) coupled with expressions of TNF-alpha (Con: 100%; STZ: 211%) and INF-gamma (Con: 100%; STZ: 205%). AAP exposure to diabetes did not alter cytokine levels but dramatically enhanced the AAP's potential to cause DNA fragmentation (AAP: 400: 302%; STZ-AA: 400: 542% of controls values). It appears that diabetes induces hepato-cellular antioxidant status and antagonizes AAP-induced hepatotoxicity and oxidative stress but fails to interfere with DNA fragmentation-related events. Induction of CYP2E1 in diabetes may be universal, and failure of a cause-and-effect relationship between oxidative stress and apoptotic death in the liver may be due to hyperglycemia or breakdown of signaling pathways.

582 MECHANISTIC TOXICOLOGY OF HYDRAZINIUM NITRATE IN PRIMARY RAT HEPATOCYTES.

S. M. Hussain, J. M. Frantz and B. Brown, 1ManTech Environmental Technology, Inc., Dayton, OH, 2AFB, Dayton, OH and 3Gro-Centers, Dayton, OH.

Hydrazine (N2H4), a simple diamine and powerful reducing agent, has been used by the US Air Force as a fuel in aircraft and propellant in rockets. Hydrazinium nitrate (H2N3O), a nonvolatile form of hydrazine, is being examined as a potential replacement for hydrazine, a known cell survivor in human hepatocarcinoma. The current study was undertaken to (1) evaluate the toxicity of H2N3O in primary rat hepatocytes in vitro, and (2) investigate whether H2N3O toxicity is mediated by oxidative stress and/or apoptosis. The effects of short-term exposure of hepatocytes to H2N3O were investigated with reference to viability, biomarkers of oxidative stress and apoptosis. Viability data showed an increase in lactate dehydrogenase (LDH) leakage, and a decrease in mitochondrial activity with increasing concentration of H2N3O. Studies of oxidative stress biomarkers showed a depletion of reduced glutathione (GSH) and an increase in oxidized glutathione (GSSG), increased reactive oxygen species (ROS) generation, lipid peroxidation and reduced catalase activity. However, there were no changes in glutathione peroxidase and glutathione redox reductase activities. Depletion of GSH and catalase activity in hepatocytes by buthionine sulfoximine (BSO) and aminotriazole, respectively, prior to exposure to H2N3O increased toxicity. It is obvious from the results obtained that H2N3O induced biochemical markers of oxidative stress. Further, studies of apoptosis revealed no significant change in total caspase activity, marginal increase in annexin V binding and partial evidences of DNA fragmentation in H2N3O treated cells. To the contrary, a decrease in mitochondrial membrane potential in H2N3O treated hepatocytes. In summary, the data suggest that short-term exposure to H2N3O is toxic to primary hepatocytes and its toxicity is attributable to oxidative stress.

583 ASSESSMENT OF APO2L/TRAIl HEPATOTOXICITY IN VITRO.


Recombinant soluble Apo2L/TRAIL induces apoptosis in a wide range of human cancer cell lines while sparing many types of normal cells and is currently being investigated as a candidate for cancer therapy. A polyhistidine-tagged version of Apo2L/TRAIL (Apo2L/TRAIL.HIS) was recently reported to induce substantial apoptosis in primary human hepatocytes, but not in rodent or monkey hepatocytes. To determine if clinical grade, non-tagged Apo2L/TRAIL exhibited activity similar to Apo2L/TRAIL.HIS, the induction of cell death by these versions was investigated in primary human and monkey hepatocytes using the MTT, Alamar BlueTR, and Crystal Violet colormetric assays. Hepatocytes were treated overnight with media, vehicle, Apo2L/TRAIL, Apo2L/TRAIL.HIS (0.01-10 mg/mL), or FasL (a hepatotaxicant serving as a positive control), followed by incubation with MTT, Alamar BlueTR, or Crystal Violet. Using all these assays, Apo2L/TRAIL.HIS and FasL induced a marked decrease in cell survival in human hepatocytes, even at concentrations as low as 0.15 mg/mL, whereas Apo2L/TRAIL induced minimal cytotoxicity, even at concentrations as high as 10 mg/mL. While the sensitivity of the Alamar BlueTR and MTT assays was sufficient to detect decreased cell survival induced by FasL, neither assay was adequate for detecting cell death by
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THE NMDA ANTAGONIST MK-801 POTENTIATES APOTOPSIS IN THE NEONATAL MOUSE BRAIN, LEADING TO HYPERACTIVITY AND LEARNING DISABILITIES IN ADULT MICE.

A. Fredriksson and P. Eriksson. Neuroscience, Uppsala University, Uppsala, Sweden and "Environmental Toxicology, Uppsala University, Uppsala, Sweden.

It is known that drugs blocking a subtype of glutamate receptors (NMDA) potentiate the naturally programmed cell death (apoptosis) occurring during the synaptogenesis which can affect learning and memory processes in animals. In humans, this period covers the last three months of pregnancy and first year after birth, which correspond to the first three to four weeks postnatally in rodents. Massive apoptosis during this sensitive period can induce neuropsychiatric disorders like ADHD (attention deficit hyperactivity disorder) in humans. Also, ethanol exposure during pregnancy can manifest as Fetal Alcohol Syndrome (FAS) with characteristic facial malformations, intellectual handicap and psychiatric disorders. In the present study 10-day-old mice were given the NMDA antagonist (+)-MK-801 0.5 mg/kg i.c. three times with 8 hours interval between the doses. One group of mice were killed 24 hours after the first injection and 10 µm thick brain sections were cut and stained using the Fluoro-Jade method. A second group of mice were tested as an adult age of 3 months for spontaneous- and d-amphetamine induced motor activity performance and learning ability in radial arm maze and Morris swim maze. The Fluoro-Jade staining showed a potentiated extensive hippocampal neurodegeneration, but also a lesser extent in frontal cortex and cerebellum of MK-801 treated animals. Neonatal MK-801 treated mice showed derangement in spontaneous motor activity (hyperactivity) and spatial learning ability. These effects could be reversed when given a low dose of d-amphetamine. Further research should be addressed to investigate anesthetic drugs used in pediatric surgery and child deliveries (ketamine and laughing gas), in order to make a proper risk assessment, regarding dose-effect relationship and possible drug interactions.

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PCBs ALTER APOTOPSIS IN PRIMARY CULTURES OF HIPPOCAMPAL BUT NOT CORTICAL NEURONS.

A. S. Howard and P. J. Len. Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.

Perinatal exposure to PCBs has been linked to cognitive dysfunction, however, the mechanism(s) underlying this effect are not known. During normal brain development, apoptosis occurs in a region and time specific manner and alteration of normal patterns of apoptosis is associated with deficits in neural function. Therefore, we investigated the possibility that PCBs alter apoptosis in neuronal cell types critical to cognitive function. Primary cultures of cortical and hippocampal neurons were treated for 48 hr beginning on day 5 in vitro with varying concentrations of Aroclor 1254 (0.1 - 10 µM), and apoptosis was assessed using the Hoechst stain. Aroclor 1254 (10 µM) significantly increased the percentage of apoptotic cells in hippocampal but not cortical cultures, suggesting that hippocampal neurons are more vulnerable to PCB induced apoptosis. To examine the role of the arylhydrocarbon receptor (AhR) in PCB induced apoptosis, we compared effects in hippocampal and cortical cultures treated with varying concentrations of 0.01 to 1 µM of either PCB 47 or 77, which bind the AhR with low and high affinity, respectively. PCB 47 (1 µM), but not PCB 77, increased apoptosis in hippocampal neurons; neither genamer altered apoptosis in cortical cultures. These data suggest that the AhR does not mediate the effects of PCBs on apoptosis. Further studies indicated that the pan caspase inhibitor z-VAD-FMK (60 µM) inhibited increased apoptosis in hippocampal cultures exposed to Aroclor 1254 and PCB 47. However, fluorometric analysis of caspase-3 enzymatic activity indicates that caspase-3 is not activated in hippocampal neurons following PCB exposure, suggesting that caspases or processes other than caspase-3 mediate PCB induced apoptosis. In summary, our data suggest that alterations in regional profiles of apoptosis subsequent to PCB exposure may be an important mechanism underlying PCB effects on cognitive development.

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MITOCHONDRIAL EVENTS IN 6-HYDROXYDOPAMINE-INDUCED APOTOPSIS: IMPLICATIONS FOR PARKINSON'S DISEASE.

L. Li and A. L. Njimba. Anatomy, Case Western Reserve University, Cleveland, OH.

Parkinson's disease (PD) is a neurodegenerative disorder characterised by a loss of dopaminergic neurons in the substantia nigra pars compacta. 6-Hydroxy-dopamine (6-OHDA) is a neurotoxic that mimics nigral degeneration in vivo and in vitro. The purpose of this study was to characterize mitochondrial changes occurring during 6-OHDA toxicity. Human neuroblastoma SH-SY5Y cells were exposed to 6-OHDA (10 µM) and 100 µM) which caused time and dose-dependent apoptotic death. After 2, 4, and 7 h exposure to 100 µM 6-OHDA, apoptosis was 3 ± 0.5%, 15 ± 4.9%, and 30 ± 2.5%, respectively, as assessed by Annexin-V staining. Loss of viability of untreated cells was 0 ± 0.2% after 7 h. Annexin-V staining paralleled caspase-3 activation, measured by cleavage of the fluorogenic caspase-3 substrate Ac-DEVD-APC. To investigate the temporal relationship between changes of mitochondrial membrane potential and cytochrome c release, SH-SY5Y cells were transiently transfected with cDNA cytochrome c-EFP and loaded with membrane potential-indicating fluorophore, tetramethylrhodamine methylster (TMRM, 250 nM). Confocal microscopy revealed a 30 ± 6.5% and 97 ± 1.7% decrease in TMRM fluorescence 2 and 4 h after 6-OHDA compared to untreated cells. The pattern of cytochrome c-EFP fluorescence was not changed after 2 h exposure to 6-OHDA, but after 4 and 6 h, the percentage of diffuse cells (cytochrome c redistribution from mitochondria to the cytosol) was 10% and 48%, respectively. Similar results were obtained by immunochemical analysis of native cytochrome c. These results indicate that mitochondrial depolarization is an early event in 6-OHDA-induced apoptotic death in SH-SY5Y cells. Furthermore, release of proapoptotic cytochrome c release is likely the consequence, not the cause, of mitochondrial depolarization. Thus, manipulations to prevent mitochondrial dysfunction may protect against 6-OHDA-induced death in dopaminergic neuroblastoma cells. Supported by NS39469.

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DIFFERENTIAL ROLE OF CELLULAR FACTORS IN THE MEDIATION OF CYANIDE INDUCED NECROTIC OR APOTOTIC DEATH.

K. Prabhakaran. L. Li, J. L. Borowitz and G. E. Isom. MCMC, Purdue University, West Lafayette, IN. Sponsored by: L.Li.

Cyanide, a well known neurotoxin, damages neurons by several mechanisms including peroxide formation, elevation of intracellular calcium and blockade of oxidase metabolism. Cyanide at the concentration range of 100-400µM induced apoptotic cell death in primary cultured cortical neurons characterized by positive TUNEL staining. After the same dose of cyanide, mesencephalic (MC) cell cultures underwent necrosis as confirmed by propidium iodide staining. It was observed that generation of intracellular oxidant species and alterations in mitochondrial functions initiate both modes of cell death. A number of intracellular factors such as caspase, Bcl-2 family members and mitochondrial pre-apoptotic factors play a role in regulation of cellular responses to the toxic insult. Initial studies show that cyanide (100-400µM) in cortical cells resulted in a significant increase in caspase activity and increased cytosolic cytochrome c. No significant changes were observed in nitric oxide synthase (NOS) and superoxide dismutase (SOD) activity. Prior treatment of cells with Z-VAD (non specific caspase inhibitor) inhibited the cyanide-induced increase in caspase activity as well as the apoptosis, suggesting that caspase mediates the cell death in cortical cells. On the other hand MC cells responded with an increased NOS activity and a significant reduction in SOD activity. Pretreatment with L-NAME (NOS inhibitor) greatly reduced cyanide-induced cell death. Thus nitric oxide appears to play a significant role in cyanide-induced oxidative stress in MC cells, but not in cortical cells. Furthermore the anti-cell death factor Bcl-2 was over expressed in MC cells after cyanide treatment suggesting a role for this protein in cyanide induced necrosis. Increased levels of nitric oxide and excess Bcl-2 may favor cyanide-induced necrosis in MC cells whereas cortical cell apoptosis caused by cyanide may involve a mitochondrial pathway. (Supported by NIH grant 01410).

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CYANIDE-INDUCED REACTIVE OXYGEN SPECIES GENERATION AND ELEVATED CYTOSOLIC CALCIUM UP-REGULATE CYCLOOXYGENASE-2 EXPRESSION IN CORTICAL CELLS.

L. Li, K. Prabhakaran, J. L. Borowitz and G. E. Isom. MCMC, Purdue University, West Lafayette, IN.

The role of cyanide-induced reactive oxygen species (ROS) generation in cyclooxygenase-2 (COX-2) expression is unclear. COX-2 is an inducible enzyme involved in arachidonic acid metabolism, which generates ROS as side products. It has been shown that ROS up-regulates COX-2 expression. In the present study, the role of
ROS generation in cytosolic-up regulation of COX-2 and apoptosis was determined. After treatment with cyanide (50-300μM) there was a prompt increase in intracellular ROS generation. Induced COX-2 expression followed ROS generation, showing that ROS regulate COX-2 expression. Pretreatment of cells with antioxidants (PBN, SOD or CAT) inhibited cyanide-induced COX-2 expression as shown by western blotting and RT-PCR. Direct stimulation of cells with H₂O₂(100-200μM) for 4h induced a dose-dependent increase of COX-2 and apoptosis (24h) similar to cyanide. Cyclopentine A, a specific blocker of mitochondrial membrane potential and permeability transition, blocked cytosolic-induced ROS generation and COX-2 expression. When cyclopentine A was added at 30 min after cyanide incubation, the effect on COX-2 expression and apoptosis was abolished. Measurement of PGE₂, a metabolite of COX-2, showed that pretreatment with antioxidants (PBN, SOD or CAT) reduced cyanide-induced COX-2 activity. When added at 30 min after cyanide incubation, 50% still inhibited neuronal apoptosis, indicating that ROS generated from COX-2 metabolism plays a key role in the apoptosis. The results from western blot and RT-PCR showed that Ca²⁺ is a mediator in cyanide-induced COX-2 up-regulation. Cyanide did not increase COX-2 expression when cells were incubated with cyanide in the Ca²⁺-free medium. BAFASTA, a membrane permeable chelator of Ca²⁺, blocked cytosolic-induced COX-2 expression and apoptosis. These results show that cyanide increases intracellular ROS and free-Ca²⁺ to mediate up-regulation of COX-2 and the sequent ROS evolving from COX-2 metabolism play a role in cyanide-induced apoptosis. (Supported by NIH grant ES 04140).

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THE ROLE OF CASPASE-3 III INHIBITION IN METHAMPHETAMINE-INDUCED ALTERATIONS IN P53 AND BCL-2 EXPRESSION: CORRELATION WITH DOPAMINERGIC NEUROTOXICITY.


Methamphetamine (METH) is a widely used drug of abuse. It causes long-lasting reductions in dopamine (DA) content, tyrosine hydroxylase activity and dopamine transporters in the striatum. It also produces oxidative stress by generating reactive oxygen and nitrogen species. METH has been reported to upregulate p53 and downregulate bcl-2 expression in striatum of mice after repeated doses. Caspase-3 is a downstream effector involved in the dissipation of the nuclei. Activation of Caspase-3 follows the onset of apoptosis. In the present study, we studied the role of caspase inhibition on METH-induced alterations in the expression of proteins p53 and bcl-2 in mice. Adult male C57 mice (n=18/group) were treated with 4 x 10 mg/kg of METH at 7 points. Other groups of mice also received a specific Caspase-3 inhibitor (CHI, 0.5 mg/kg) or a general caspase inhibitor (GC, 0.5 mg/kg) along with METH or alone. Saline treated animals served as control. Animals were sacrificed 72 h after the last dose of METH. METH administration resulted in significant up-regulation of p53 and down-regulation of bcl-2 in mice striatum. GC failed to protect against these effects, while CHI significantly protected against METH-induced genetic changes in the mice striatum. METH also resulted in the significant depletion of DA and its metabolites DOPAC and HVA. CHI provided a significant attenuation whereas GC failed to provide any protection against dopaminergic neurotoxicity. Therefore, the present data demonstrated that METH-induced dopaminergic neurotoxicity caused genetic alterations, which can lead to the onset of programmed cell death, however, it can be protected by specifically targeting the downstream effector action of Caspase-3.

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ROLE OF MITOCHONDRIAL BCL-2 PROTEIN AND DOPAMINE IN ENVIRONMENTAL CHEMICALS-INDUCED DOPAMINERGIC CELL DEATH.

M. Kishrawy, V. Anantharam, S. Kaul and A. G. Kambhaghat. Biomedical Sciences, Iowa State University, Ames, IA.

Several epidemiological studies have implicated chronic exposure to certain pesticides and metals in the etiology of Parkinson's disease. Manganese and dieldrin are two prominent environmental toxicants that have been associated with Parkinson's disease. We previously demonstrated that dieldrin and methylycyclopentadienyl manganese tricarbonyl (MNT) share similar cell death signaling process and induce apoptosis in dopaminergic cells. In the present study, we have further characterized biochemical mechanisms underlying dieldrin- or MNT-induced selective dopaminergic toxicity. When exposed acutely, both dieldrin and MNT show relatively selective cytoxicity to dopaminergic PC12 cells with EC₅₀ of 142μM and 200μM, respectively, EC₅₀ of dieldrin to HCNT-2 cells (human cortical neuronal cells) and α-TC (pancreatic β cells) is 29.6μM and 35.1μM, respectively, and EC₅₀ of MNT in striatal GABAergic cells is 577μM. The dieldrin- or MNT-induced generation of reactive oxygen species (ROS) and apoptotic cell death are significantly attenuated by pre-treatment with α-methyl-p-tyrosine or deprenyl, indicating that the presence of dopamine may, in part, contribute to susceptibility of dopaminergic cells to these environmental neurotoxins. Furthermore, using Bcl-2 transfected PC12 cells, we have demonstrated that these chemicals may target mitochondria and initiate apoptotic cell death signaling cascades. Reduction of mitochondrial membrane potential, caspase-3 activation and DNA fragmentation are significantly (p<0.01) attenuated in Bcl-2 overexpressed PC12 cells following acute exposure to dieldrin (10-100μM) or MNT (10-200μM). Together, our results suggest that dopaminergic release and mitochondrial dysfunction may serve as a common mechanism underlying environmental factor-induced dopaminergic degeneration. Supported by NIH grant ES 10586.

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ACTIVATION OF CASPASE-3 BY DIETHYLTHIODICARBAMATE (DDC) IN RAT HIPPOCAMPAL ASTROCYTES WITH OR WITHOUT GLUTATHIONE.

J. A. Mocker-Audrain and L. D. Tombetta. Pharmaceutical Sciences, St. John's University, Jamaica, NY.

Diethyldithiocarbamate (DDC), is a carbamyl derivative and potent metal chelator. It has been shown to bind and redistribute copper in a lipophilic complex to many organs including the central nervous system. We have previously demonstrated by TUNEL and DNA laddering techniques that 350μg/ml and 350μg/ml DDC induced apoptosis or programmed cell death in rat hippocampal astrocytes treated for 1hr. Caspases, also known as interleukin-1β-converting enzyme (ICE), are cysteine proteases that have been implicated in apoptosis. The activation of the caspase cascade requires the activation of initiator caspases by a toxic insult which in turn activates effector caspases. Caspase-3 cleaves a set of vital proteins and executes the cascade of events leading to the apoptotic degradation phase. Glutathione plays a major role in the antioxidant defense system. It has been documented to participate in the degradation of xenobiotics. It has also been documented in the literature that glutathione maintains cytoskeletal integrity by stabilizing intracellular sulhydryl status. Previous experiments have shown that glutathione (10nm) post-treatment protects against DDC insult. The purpose of this experiment was to determine whether caspase-3 may be induced following the treatment with DDC and whether glutathione prevents caspase-3 activation. Rat hippocampal astrocytes known in Dulbecco's modified Eagle's essential media were treated with 35 and 350 μg/ml DDC at subconfluency for 1 h. Astrocytes were then left to recover with or without 10μM glutathione in DMEM for 48 h at which time caspase-3 activity was assayed. Preliminary studies suggest that caspase-3 is activated in astrocytes treated with 35 and 350 μg/ml DDC. Glutathione post-treatment prevents the activation of caspase-3 in both treated groups.

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CASPASE-3 MEDIATED PROTEOLYTIC CLEAVAGE OF PKCβ PLAYS A CRITICAL ROLE IN METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MNT)-INDUCED APOTOTIC CELL DEATH IN MESENCEPHALIC CLONAL CELLS.

V. Anantharam, M. Kitazawa, S. Kaul, S. Ranade, M. L. Kirby and A. G. Kambhaghat. Biomedical Sciences, Iowa State University, Ames, IA.

The cellular mechanisms underlying selective dopaminergic degeneration in PD remains elusive. Oxidative stress has been suggested to contribute the pathogenesis of PD. Additionally, environment exposure to certain pesticides and transition metals such as manganese have a positive association with increased incidences of PD. We characterized oxidative stress dependent cellular events by exposing an immortalized rat mesencephalic dopaminergic cell line (1RbAN10) to an organic form of manganese compound, methylycyclopentadienyl manganese tricarbonyl (MNT). Exposure of MNT (30-100 μM) resulted in a rapid increase in generation of reactive oxygen species (ROS) within 5-15 min and was followed by release of mitochondrial cytochrome C into cytoplasm. Cytochrome C then activated a proapoptotic cysteine protease, caspase-3 (G-fod) in a time- and dose-dependent manner. Interestingly, we also found that MNT exposure induces a time- and dose-dependent proteolytic cleavage of native protein C9CKβ(72-74 KDa) to yield 41KDa carboxy terminally active 38KDa regulatory fragments. Pretreatment with caspase inhibitors (Z-DEVD-FMK or Z-VAD-FMK) blocked MNT-induced proteolytic cleavage of PKCβ, indicating that cleavage is mediated by caspase-3. Attenuation of ROS generation, caspase-3 activation and PKCβ activity prior to MNT treatment almost completely suppressed DNA fragmentation. Additionally, overexpression of catalytically inactive PKCβ (dominant negative mutant) prevented MNT-induced apoptosis in immortalized mesencephalic dopaminergic cells. Preliminary data in ovine rat brain slices exposed to MNT indicated proteolytic cleavage of PKCβ within 3 hr of treatment. For the first time, these data demonstrate that caspase-3 dependent proteolytic activation of PKCβ plays a key role in oxidative stress-mediated apoptosis in dopaminergic cells following exposure to an environmental neurotoxic agent.
METHYLMERCURY INTERACTS WITH MUSCARINIC RECEPTORS DURING NEURONAL DEATH IN RAT CEREBELLAR GRANULE CELLS.

S. A. Loiselle, T. L. Limke and W. D. Ashton, Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

Cerebellar granule cells are highly susceptible to MeHg-induced neuronal death both in vivo and in vitro. One hallmark of this neuronal death is severe Ca2+ deregulation, which contributes to delayed neuronal death in primary cultures of rat cerebellar granule neurons. Within granule cell bodies, the SER represents a high affinity, low capacity Ca2+ store which may contribute to MeHg-induced Ca2+ elevations by release of Ca2+ through inositol-1, 4, 5-trisphosphate (IP3) receptors. Because this Ca2+ can be released through activation of M3 muscarinic acetylcholine receptors, we examined whether inhibition of these receptors is cytotoxic during MeHg exposure. We used neonatal rat cerebellar granule neurons in primary culture to measure cell viability using a calcium-estadiol homodimer live/dead cytotoxicity assay. Previous results indicated that down-regulation of muscarinic and IP3 receptors with 24 hr brehatichol (1 mM) was neuroprotective (Martinez et al., The Toxicologist 30, 184, 2001), suggesting a role for muscarinic and/or IP3 receptors in MeHg-induced cell death. In our experiments, the protection afforded by brehatichol was not affected by the nicotinic receptor antagonist dihydro-β-erythroidine, indicating that protection was through the muscarinic receptors. However, application of atropine alone (10 mM) did not attenuate cell death caused by 0.2-1.0 μM MeHg. Inhibition of Ca2+ release through the IP3 receptor with 1 mM xestropogonin C was also not protective. Interestingly, 24 hr application of 1 mM xestropogonin C or acute application of the phospholipase inhibitor U73122 (1 μM) alone caused significant cell death, highlighting the importance of SER Ca2+ signaling pathways in maintenance of cell viability. These results suggest that the protection afforded by 24 hr BCh treatment occurs through muscarinic receptors, and that disruption of this pathway is neurotoxic in cerebellar granule neurons. Supported by NIEHS grants RO1 ES03259 and T32 ES07255.

THE 2-NITROIMIDAZOLE RADIOSENSITIZER CI-1010 INDUCES SELECTIVE APOPTOSIS IN THE PHOTOSENSITIZING EFFECTS OF C57BL/6 MICE.

T.J. Miller1, L. A. Dahloff2 and M. A. Philbert3. Environmental Health Sciences, University of Michigan, Ann Arbor, MI and Pfizer Global Research and Development, Ann Arbor, MI.

CI-1010, a bifunctional nitroimidazole, produces selective apoptosis in photoreceptors in vivo. CI-1010 triggers opening of the mitochondrial permeability transition (MPT) pore in SYSY neuroblastoma cells following oxidation of the intracellular space. This study investigates in vivo mechanisms of CI-1010-induced retinal toxicity. Adult C57BL/6 mice treated with a single intraperitoneal injection (1000 μmol/kg) were necropsied at 4, 2, 24, 48, 72, and 96th following treatment. Morphological evaluation by light microscopy reveals retinal degeneration by 96h localized to the photoreceptor (PRL) and outer nuclear layer (ONL) of the retina. By 12h, the PRL appears vacuolated, compressed, and poorly organized. The pattern of retinal degeneration is time-dependent and characterized by thinning of both retinal layers due to loss of photoreceptor segments and nuclei, culminating in the collapse of the PRL. Apoptotic bodies are visible in the ONL by 72h and many nuclei label with (TdT) mediated 3'-OH fluorescent end-labeling. Western blot analysis of retinal homogenates from treated mice show decreased expression of the anti-apoptotic protein, Bcl-xL, at 4h, remaining less than control at all timepoints. Bcl-xL is responsible for stabilization of the MPT pore in the closed position. Reduced Bcl-xL expression may destabilize the MPT pore in the PRL. Analysis of caspase 9 and 8 activation suggests involvement of both the mitochondrial and non-mitochondrial pathways by 24h. Caspase 3 is active at 72h. The precise role of mitochondria and caspase activation in CI-1010-induced neuronal cell death is unknown. However, these results suggest that retinal apoptosis induced by CI-1010 is mediated by caspase-3 activation subsequent to the execution of a mitochondrial pathway of cell death.

959 BCL-XL INHIBITS LEAD-INDUCED ROD PHOTORECEPTOR APOPTOSIS BY BLOCKING THE INCREASE IN MITOCHONDRIAL CONTACT SITES.

D. A. Fox1, G. A. Perkins1, L. He1, A. T. Poblan2, M. H. Ellisman3 and J. B. Harris3. U. Houston, Houston, TX, UCSF, San Diego, CA. 1UNC, Chapel Hill, NC and 3UT MD Anderson, Houston, TX.

Photoreceptor apoptosis and visual deficits occur in man and animals with inherited and chemical-induced retinal degenerations, and retinal diseases and injuries. Rod-selective apoptosis is produced by developmental lead exposure in mice and by in vitro lead and/or calcium; the latter activates the cytochrome c-caspase cascade of apoptosis (IBC, 2000, SOT). This study used wild type and transgenic mice overexpressing Bcl-xL in photoreceptors to examine the role of mitochondria in lead-induced rod apoptosis. Newborn mice were raised by dams drinking water (controls) or 0.15% lead acetate solution. At weaning (BP = 26 μg/kg) studies of retinal mitochondria were conducted in all four groups using three-dimensional electron microscopic tomography. JCS staining of cytochrome c (cyclo) and caspase assays and oxygen consumption. There were no differences between the four treatment groups for 5 of 7 different measurements of rod mitochondrial substructures (crista width, cristae junction diameters, outer (OMM)-inner membrane width, mitochondrial contact site width, contact site diameters) and no physical disruptions of the OMM or swollen mitochondrial membranes. Lead-induced rod apoptosis was accompanied by calcium overload, increases in mitochondrial contact site area and density, decreases in rod mitochondrial respiration and membrane potential (depolarization), cyc c release and caspase activation. Bcl-xL overexpression completely blocked the lead-induced increase in contact sites and all apoptotic events, except calcium overload, and maintained normal rod respiration. These results suggest that Bcl-xL is located and functions at mitochondrial contact sites to block the opening of the permeability transition pore. These findings have relevance for therapies in human and animal retinal and neuronal degenerations where lead exposure, calcium overload and/or mitochondrial dysfunction occurs. Supported by NIH Grants ES03183, ES07088, RR04050, NS14718, K125444.

596 MERCURIC CHLORIDE-INDUCED PROGRAMMED CELL DEATH IN MOUSE LEYDIG CELL LINE (LF6G).


There is strong evidence that exposure to heavy metals, in general, and mercurial compounds in particular, impact the male reproductive system. A multicellular study, recently, completed our laboratory, demonstrated that mercuric chloride causes a reduction of fertility indices, reproductive indices and testosterone level. Recent studies have demonstrated that germ cell apoptosis increases after testosterone withdrawal from the testes. The present study was undertaken to determine whether testosterone reduction associated with exposure to mercuric chloride occurred due to Leydig cell death by the apoptosis. Mouse Leydig cell line (LF6G) was exposed in Dulbecco’s modified Eagle’s medium (DMEM) to mercuric chloride concentrations 0.0, 3.75, 7.5, 15 or 30 μM. Viability of Leydig cell was tested using trypan-blue exclusion assay after 1, 6, 12 and 24 hours of mercuric chloride exposure. Cells in control, 2.75 and 7.5 μM groups had a viability of approximately 96%, regardless of exposure time. In contrast, the viability of the cells in 15 and 30 μM treatments gradually declined to approximately 85% and 60% respectively, after 24 hours of exposure. Agarose gel electrophoresis and ligase-mediated polymerase chain reaction (LM-PCR) were used to assess the presence of apoptosis. The nucleosomal DNA fragmentation was quantitated visually and by using Fluor-S Multimager. The intensity of DNA ladder in exposed cells increased significantly in a concentration- and time-dependent manner when compared to that of the controls. These results suggested that mercuric chloride caused a significant apoptosis in a Leydig cell line and the mechanism of mercuric chloride-induced testosterone level reduction in vivo may be related to the induction of apoptosis.

597 EVIDENCE OF MITOCHONDRIA-ASSOCIATED SIGNALING IN TESTICULAR GERM CELL APOPTOSIS IN ADULT C57BL/6(B6) MICE AND SPRAGUE-DAWLEY RATS EXPOSED TO MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP).

C. J. Giovanni, P. Sawhney and J. H. Rickburg. College of Pharmacy, The University of Texas at Austin, Austin, TX.

The Fas signaling pathway has been previously characterized as a key mediator of germ cell apoptosis, particularly after toxicant-induced Sertoli cell injury. Recent reports have indicated that cell-damaging agents can stimulate apoptosis through a "stress-induced" mitochondria-regulated signaling pathway. In stress-induced apoptosis, the release of cytochrome C (initiated by the pro-apoptotic proteins Bak and Bax) appears to be a critical event. Cytochrome C release results in the activation of caspase-9 and eventual cell death. Interestingly, caspase-9 activation has been shown to increase the release of cytochrome C by truncating Bid to tBid, which can then oligomerize with Bax or Bax causing the release of cytochrome C. We propose that the mitochondria-associated signaling system is important in the initiation of apoptosis after MEHP-induced germ cell injury and may act in conjunction with the Fas signaling system. Adult C57 mice and adult Sprague-Dawley rats were exposed to an acute dose of MEHP (1 mg/kg p.o.). Western blot analysis demonstrated early increases (by 3 h post-MEHP exposure) in cytosolic cytochrome C and subsequent increases in active caspase-9 and caspase-3 in mice. Immunohistochemistry (IHC) on both frozen tissue sections and fresh testis touch
preparations revealed gene-specific protein expression and/or localization of Box and caspases in rat testes. These findings shed light on stress-related signaling mechanisms. Proteins were isolated using various conditions, including buffer changes and substrate additions. (Supported by R01ES09145, Trained Grant ES07247, NIEHS Center Grant 07784).

598 PULSATILLA NIGRICANS (HOMEOPATHIC DRUG) TREATMENT INCREASED APOPTOSIS AND ATRIA IN RAT OVARIAN FOLLICLES ALONG WITH DECREASED DNA LEVELS.


Pulsatilla nigricans is an age-old homeopathic drug prescribed for various reproductive ailments especially in women. The drug contains an alkaloid, ammonium, anemoninc acid and potassium sulphate. The mode of action of the drug and the effect of different doses (Potencies) is not well understood. Hence the present study was under taken to evaluate the effects of Pulsatilla nigricans on parametrical mechanisms involving follicular atraia along with quantification of DNA. Female albino rats of wistar strain were administered with different potencies of the drug orally on alternate days for a period of four weeks. The biochemical study revealed that 200c potency has shown a significant decline in the DNA content of the ovary. This was indicative of low rate of protein synthesis and actinogenes stress in the ovary. Likewise histological studies revealed consistent follicular atraia with prolonged rhythmic and metostrous phases when treated with 200c potency. The drug with 200c potency had shown a maximum decline in the DNA levels possibly because of increased DNA fragmentation when compared with the other potencies. The same potency showed maximum follicular atraia and significant number of apoptotic cells (P = 0.005); Ovarian cycle in general become a complex networking of tissue growth and differentiation of complex networking of programmed cell death / atresia. In conclusion, our findings suggested that 200c potency of Pulsatilla is a strong antifertility compound, which can be used as a safe and cheaper contraceptive in the developing countries.

598A DAMAGE OF DNA IN CULTURED SKIN CELLS BY LOW DOSES OF AS(III).

L. L. Bergstrom, Y. Chong and J. Niiza. Environmental Health Sciences, University of Michigan, Ann Arbor, MI.

We have previously established the higher toxicity of As(III) than that of As(V) for nucleated cells including skin cells on integrity of the cell membrane, the rate of DNA, RNA and protein synthesis in primary human skin cultures. In this study we demonstrated that: a) exposure of human keratinocytes (HK) cultures to As(III) produces a dose-dependent decrease in protein concentration as well as cell viability (50% cytotoxicity) at 500μg/L. b) A concentration-dependent increase in the rate of cell proliferation was observed in HK cultures treated with As(III). c) Exposure to As(III) decreased the amount of DNA content (60% decrease) in HK cultures treated with As(III). d) Exposure to As(III) decreased the amount of DNA content (60% decrease) in HK cultures treated with As(III). The results of these experiments suggest that As(III) has a significant impact on DNA integrity in skin cells and may contribute to the development of skin cancer.

599 INDUCTION OF CYP ISOFORMS BY PESTICIDES AND DEET AS MONITORED BY THE BDNA ASSAY.

T. L. Rose*, N. J. Cherrington, J. Tang*, Y. Cao*, D. P. Hatley*, C. D. Klauser* and E. Hubicki*, Toxicology, North Carolina State University, Raleigh, NC and Medical Center, University of Kansas, Kansas City, KS.

Drug-drug interactions often occur when important drug metabolizing enzyme levels are altered as a result of exposure to another drug. As a consequence of this phenomenon, drugs are routinely screened for their ability to induce cytochrome P450 enzymes, one of the more important drug metabolizing enzyme families. Pesticides are also known to induce cytochrome P450 enzymes in humans. Studies of gulf war veterans suggest that pesticide exposure may have had negative impact on veterans. Our previous rodent studies confirm that pesticides and insect repellants such as DEET can induce enzymes important in pesticide activation and/or detoxification reactions. To determine possible pesticide interactions in humans, cultured human hepatocytes were exposed to 200μM warfarin and DEET also induced DEET caused the increase in CYP3A4, 2B6 and 2A3. Since our previous studies using human lymphoblast cell lines demonstrated the importance of CYP2B6 in the activation of chlorpyrifos to chlorpyrifos-oxon, this work suggests that pesticides and repellants such as DEET have the potential to increase and/or decrease pesticide susceptibility.

600 FORMATION OF UBIQUITIN-CYTOCHROME P450 3A CONJUGATES IN ISOLATED MICROSOMES.


A novel microsome system is described that conjugates CYP3A and ubiquitin to form high molecular weight (HMW) complexes. The induction of this process in isolated microsomes appeared to be dependent upon extended in vivo treatment with potent CYP3A inducers. Treatment of the microsomes to remove photolabile compounds of cytochrome P450 did not prevent the formation of the HMW CYP3A complex. Exposure to CYP3A induced ubiquitin conjugates indicated that this protein was complexed with ubiquitin. Even so, the conjugation reaction did not require addition of ubiquitin. ATP, Mg++, or cytosol and, as such, was unlikely to be dependent upon the ubiquitin activating enzyme E1. Furthermore, mass spectrometric analysis of tryptic peptides strongly suggested that the HMW CYP3A was in nodular excess to ubiquitin. This result suggested that the HMW CYP3A ubiquitin conjugates may be conjugated to itself or a diffuse pool of already ubiquitinated proteins present in the sample. CYP3A substrate blocked formation of the HMW CYP3A in the incubated microsomes. These results suggest that extended induction of CYP3A expression results in the activation of microsomal factors that catalysed the formation of HMW CYP3A conjugates. This conjugation reaction may be related to the substrate-dependent stabilization of CYP3A observed in vivo but appears to be distinct from the classical ubiquitination pathway.

601 SAFETY EVALUATION OF CHRONIC DIETARY EXPOSURE TO 3, 5-DINITRO-METHANE (DIM) IN SPRAUCE-DAWLEY RATS.

D. A. Lebel*, O. R. Hedstrom* and D. E. Williams. Environm., & Mol. Toxicology, Oregon State University, Corvallis, OR and College of Vet. Med., Oregon State University, Corvallis, OR.

Indole-3-carbinol (I3C), a naturally occurring component of the human diet, is found in significant concentrations in cruciferous vegetables. In an acidic environment, such as the stomach, I3C forms conjugation products, one of the most abundant of these being DIM. DIM is marketed as a dietary supplement but little is known about the safety of long-term exposure. Male and female Sprague-Dawley rats were fed either control diet, diet containing the proposed human dose of Bioresearcher's DIM equaling 6.45 mg/kg/day, or diet containing ten times the proposed human dose equaling 64.5 mg/kg/day. The experiment was terminated after 3 or 12 months of exposure. Small animal chemistry panels were performed along with histopathological examination of the liver, kidneys, adrenals, and colon of all animals, the testes, prostate, and epididymus for the males, and the ureters and ovaries for the females. The total cytochrome P450 (CYP) content of the liver was determined along with the levels of the specific isoforms CYP1A1 in liver and colon and CYP3A2 in the liver. There were no differences between groups in blood chemistry. Most notably no increases were seen in markers for liver or other tissue damage. Tissue examination yielded no observable differences grossly or histologically between groups, although a high number of bile ducts and bile canaliculi were found throughout the kidney tissue of all animals. Total liver DNA levels were induced (280%) only in males. Slight induction of CYP1A1 and CYP3A2 was seen in liver and CYP1A1 in colon for both sexes. Comparison to I3C indicates that DIM is a markedly less potent inducer of CYP1A1 in the rat. Supported by the OSU Ag. Res. Fdn.
EVALUATION OF ALBENDAZOLE SULFOXIDE AS A PROBE FOR CYP1A1 ACTIVITY.

J. Anzimma Castro, D. M. Escobar García and J. J. Espinosa Aguirre. Genetics and Toxicology, Universidad Nacional Autónoma de México, Mexico City, Mexico. Sponsor: M. Gomendio.

Albendazole Sulfoxide (ABZSO) is the active metabolite of the anthelmintic drug Albendazole (ABZ). Previous reports on ABZ pharmacokinetics in rats (pretreated with different cytochrome P450 (CYP) inducers) suggest that ABZSO is metabolized to Albendazole Sulfoxide (ABZSN) by cytochrome P450 3A1 (CYP3A1). In order to test this compound (ABZSO) as a probe to evaluate CYP1A1 activity in rat, three experimental groups were used: a) Control group; b) Induced group; and c) Induced group with ABZSO. The groups were treated p.o. with a single dose of Albendazole Sulfoxide (20 mg/kg, suspended in corn oil). Groups b) and c) were previously treated with a CYP1A1 inducer (β-naphthoflavone) and a CYP1A1 inhibitor (3-methylcholanthrene), respectively. After treatment with ABZSO, plasma levels of ABZSO and ABZSN were evaluated through 18 h with HPLC. CYP1A1 activity (Ethoxyresorufin O-deethylase) was measured in liver microsomes from animals belonging to each experimental group. Correlations between pharmacokinetic parameters and hepatic CYP1A1 activity will be presented.

QUININE METABOLISM AS A PROBE FOR THE EVALUATION OF RAT HEPATIC CYP1A1 AND CYP3A.


The in vivo metabolism of quinine was investigated with 15 recombinant rat cytochrome P450 enzymes. It was found that the CYP3A1 enzyme produced 3-OH quinine as the major product (90% of all metabolites detected) of quinine bioactivation. The recombinant CYP3A2 was active in metabolizing quinine into 3-OH quinine but with a rate approx. 30% of that for CYP3A1. No significant formation of 3-OH quinine was found in the experiments with other 13 recombinant rat CYP enzymes. It was found that the CYP3A1 enzyme produced O-demethylquinine as the major metabolite of quinine with no significant formation of 3-OH quinine. No significant formation of O-demethylquinine was found in the experiments with either CYP3A1, CYP3A2 or CYP2J1, as well as with 11 other recombinant rat CYP. In liver microsomes incubated with quinine, the rates of formation of 3-OH quinine were 0.26, 0.32, 22.0 and 1.0 nmol/mg protein for female control, MC- and DEX-induced and male control, respectively. Among the microsome preparations tested the only female MC-induced microsomes were active in the production of O-demethylquinine. Overall, the data obtained with recombinant rat cytochrome P450 enzymes are in the good agreement with the data obtained with hepatic microsomes from various treated rats. Enzymes kinetics (Vmax, Km and Clint) determined for rat CYP1A1 and CYP3A1 indicate that both enzymes are similar active in quinine bioactivation although each produces different metabolites. This study indicates that two different products of quinine bioactivation could be used as suitable probes for the in vivo and/or in vivo evaluation of CYP1A1 and CYP3A metabolic activities. (Supported by GM 44982 and ES 05022)

STRUCTURE-ACTIVITY RELATIONSHIPS (SARS) FOR IN VITRO INHIBITION OR INDUCTION OF AROMATASE CYP19 ACTIVITY BY VARIOUS PESTICIDES.


SARs were assessed for aromatase (which converts androgens to estrogens) inhibition or induction by various pesticides in H295R human adrenocortical carcinoma cells. The effects of 13 pesticides were assessed and compared to literature data for in vitro inhibition or induction by structurally similar pesticides. Among the most potent aromatase inhibitors were the fungicides imazalin and prochloraz, which both contain 5-methyl and chloroaniline, desmethylation, and the structurally similar imidazole fungicides (in order of decreasing potency): fenecomazole, desmethylation, and the structurally similar triazole fungicides thiadenzole and tridiazinone. The structurally similar triazole fungicides thiadenzole and the triadiazinone were almost inactive in H295R cells, as were the pyrimidine fungicides fenamidone (a known microsomal aromatase inhibitor) and nufamidone, probably due to the limited cellular uptake of these relatively polar compounds. Enzyme kinetic analyses indicated that the imidazole fungicides were uncompetitive, whereas the triazole fungicides were competitive inhibitors of aromatase with K_i values in the low micromolar range. The triazole herbicides atrazine, simazine, and propazine, the structurally similar vinclozolin and to a lesser extent the hydroxylated triazole fungicide dicloflurenol were inducers of aromatase activity. Atrazine and vinclozolin act by increasing intracellular cAMP levels, possibly through inhibition of phosphodiesterase activity, suggesting this enzyme as a target for SAR analysis of aromatase inducers in H295R cells. These SARs may be useful to identify chemicals with the potential to disturb the androgen/estrogen balance through an interaction with aromatase.

HYPOXIA DIFFERENTIALLY MODULATES CYTOCHROME P450 EXPRESSION IN NEWBORN AND ADULT RATS: IMPLICATIONS FOR OXYGENATED MEDITATED LUNG INJURY.


Supplemental oxygen is frequently administered to infants having pulmonary insufficiency. Newborn rats showed increased tolerance to hyperoxic lung injury than adult rats. In this study, we tested the hypothesis that exposure of newborn or adult rats to hyperoxia would differentially regulate hepatic and pulmonary cytochrome P450 (CYP) isoforms, a phenomenon that may contribute to the increased tolerance to injury in the newborn rats. Newborn Fisher rats or adult rats were maintained in room air or exposed to hyperoxia (>95% O2) for 1-7 days, and animals were sacrificed at selected time points. Pulmonary and hepatic CYP1A1, CYP1A2, and CYP3A4 mRNA expression was determined by RT-PCR. All newborn rats survived 7 days of hyperoxic exposure. On the other hand, none of the adult rats survived beyond 60 h of hyperxia. In the newborn liver, hyperoxia markedly induced CYP1A1, CYP1A2 and CYP3A4 expression over room air control after 24-48 h of exposure, the expression of CYP1A1, but not CYP3A4, declined after 7 days. In the newborn lung, hyperoxia induced CYP1A1 and CYP4F4 expression early in the time course (1-7 days), and expression of both isoforms declined by 7 days. In adult rats, the hepatic expression of CYP1A1/2 and CYP4F4 was enhanced between 24-48 h of hyperoxia, followed by decline at 60 h. In contrast, pulmonary CYP4F4, was induced by hyperoxia only at 60 h. The sustained induction by hyperoxia of CYP1A2 and CYP4F4 genes in the newborn but not adult liver suggests an important protective role for these enzymes against hyperoxic lung injury. Furthermore, the augmentation of pulmonary CYP4F4 gene expression early in the time course of hyperoxic exposure in the newborn rat may contribute to the increased tolerance to oxygen-induced lung injury. (Supported in part by NIH grant ES09132 and by ALA.)

ALLEVIATION OF HYPOXIC LUNG INJURY IN THE RAT BY THE CYTOCHROME P450A1 INDUCER 3-METHYLCHOLANTHRENE.

B. Moschoy and W. Jiang. Pediatrics, Baylor College of Medicine, Houston, TX. Sponsor: B. Moschoy.

Supplemental oxygen administration is used extensively in the treatment of pulmonary insufficiency that is commonly observed in infants and in premature infants. We reported earlier that exposure of rats to hyperoxia for 48 h leads to induction of hepatic CYP1A1/2 enzyme activity, followed by decline at 60 h, the time point that coincides with severe respiratory distress and lung damage. In this study, we tested the hypothesis that pretreatment of rats with the CYP1A1 inducer 3-methylcholanthrene (MC) would protect animals from lung damage caused by subsequent hyperoxic exposures. We treated adult male Sprague-Dawley rats with MC (93 μmol/kg) or vehicle only, once daily for 4 days. After 1 or 15 days after MC withdrawal, the animals were either maintained in room air or exposed to hyperoxia (95% O2) for up to 60 h. Activities of pulmonary and hepatic ethynethoxyresorufin-O-deethylase, which is specific for CYP1A1, were about 30-40-fold elevated in the MC-treated rats as both 1 and 15 day time points, compared to vehicle-treated controls. Seven out of 9 control rats survived 60 h of hyperoxia. On the contrary, 12/12 MC-treated animals survived 60 h of hyperoxia. This was the case for both the 1 and 15 day time points. Vehicle-treated animals exposed to hyperoxia for 60 h had severe measurable pleural effusions (10.4 ± 2.0 g), which is an index of lung injury. In contrast, MC-treated animals after 60 hyperoxia had significantly lower pleural effusions, being 3.1 ± 1.24 (g), respectively, for 1 and 15 day time points. The decreased susceptibility of MC-treated animals to oxygen-induced lung damage, in conjunction with the sustained CYP1A1 expression in these animals, supports the hypothesis that CYP1A1 induction plays a beneficial role in hyperoxic lung injury. (Supported in part by NIH grant ES09132 and by ALA.)
607 UROPHYLLA CAUSED BY FE AND ALA IN MICE: THRESHOLD REQUIREMENTS FOR FE AND CYP1A2.

P. R. Sinclair1, 2, N. Gorman1, K. L. Ross1, H. S. Walton1, W. I. Benten1, J. G. Szakacs1, G. S. Gerhard1, T. P. Dalen1, D. W. Nebert2, R. S. Eisenstein1, and J. E. Liljenblad1. 1VAD Med. Center, White River Junction, VT. 2Biochem., Pharmacol. Toxicol., Pathology, Dartmouth Medical School, Hanover, NH. 3VA Med. Center, Salt Lake City, UT. 4Nutritional Sciences, University of Michigan, Madison, WI. 5Environmental Health Center, University of Cincinnati, Cincinnati, OH.

Previous work has implicated both CYP1A2 and Fe in experimental urophylaria (URO) accumulation caused by polyhalogenated compounds, α-aminolevulinate (ALA) and Fe. In mice, strain differences in sensitivity for urophylaria caused by ALA and Fe have been observed. We examined whether the higher sensitivity to urophylaria caused by ALA alone in SWR compared to C57BL/6 mice was due to higher endogenous levels of hepatic and intestinal CYP1A2 in SWR mice. The ALA and Fe-treated CYP1A2 (-/-) heterozygote which has CYP1A2 protein levels about 60% of the CYP1A2 (+/+) wild-type, accumulated much less URO than the wild-type, whereas Cyp1a2 (-/-) mice accumulated no URO. In C57BL/6 mice treated with ALA, increasing CYP1A2 about 2-fold by a low dose of PCB126, increased URO accumulation by 5-fold, if Fe was also given. A Dose-Response study with Fe-deter-mined in mice given PCB126 and ALA, showed that hepatic levels of Fe greater than 800 micrograms/gram weight were required to cause significant URO accumulation. Perls staining revealed that at low Fe doses, Fe accumulation was mainly in kupffer cells; with higher doses more Fe was also detected in parenchymal cells. Two indices related to the availability of iron, IRP-1 binding and accumulation of posttranscriptional regulatory iron chelate, were decreased at much lower Fe doses than those required to produce urophylaria. We conclude that while increasing CYP1A2 increases the sensitivity of C57BL/6 mice for urophylaria to that of SWR mice, merely increasing hepatic Fe to the same level does not. Other strain specific factors, especially in Fe metabolism may be involved in this process. This work was supported by the VA and NIH (ES06263, ES06321, AG14731, DK47219).

608 CYTOCHROME P450 REDUCTASE MUTANTS WITH ALTERED COFACTOR SPECIFICITY (NADPH VS. NADH).

C. L. Emirong and T. D. Porter. Graduate Center for Toxicology, University of Kentucky, Lexington, KY. Sponsor: M. Vazquez. Cytochrome P450 reductase (CPR) is responsible for electron transfer from NADPH to the cytochromes P450 and other microsomal monooxygenases. CPR has an unusually high specificity for NADPH (vs. NADH). These two cofactors differ only in the presence of a 2′-phosphate instead of a hydroxyl group (OH) on the adenosine ribose of NADPH, and thus their moiety must provide the target for cofactor discrimination. We have used site-directed mutagenesis to identify several residues that discriminate between the 2′-phosphate of NADPH and the 2′-hydroxyl of NADH: amino acids were chosen for mutagenesis based on the crystal structure of ferredoxin NADP+ reductase in conjunction with sequence alignments of NADPH-specific enzymes. Five single mutants (S596D, R597M, S596D,K602W, R597M, W677A, K602W,W677A) and four double mutants (S596D,R597M, S596D,K602W, R597M, W677A, K602W,W677A) were generated. Most notably, S596D, S596D,R597M, and S596D,K602W show a greater than 23,000-fold shift in Km value from NADPH to NADH. W677A, S596D,R597M, R597M,W677A, and K602W,W677A mutants show a higher lca (with NADH) than with NADPH. The W677A mutant shows the highest overall catalytic efficiency (lca/KM) with NADH, with a KMa of ~3000 (comparable to the wild-type enzyme with NADPH) and a Km of ~100 μM. To determine if mutations to the 2′-phosphate binding region of P450 reductase might allow activity with NADH without inhibition by NADPH, we tested K602W,W677A along with the W677A enzyme in the presence of 2.5 μM NADPH. K602W,W677A retains significant activity in the presence of 2.5 μM NADPH, a concentration that causes complete inhibition of W677A. This enzyme may be suitable for use in in vivo applications. This research was supported by NSF Grant MCB-5808636 and NIH Training Grant ES-67266.

609 GENDER DIFFERENCES IN THE METABOLISM OF ACYLONITRILE TO CYANIDE IN MICE: ROLE OF CYTOCHROME P4502E1 (CYP2E1) AND EPOXIDE HYDROLASE (EH).

B. Chabais, H. Wang, and B. J. Gishiew. 1NIH/NIEHS, Research Triangle Park, NC. 2University of North Carolina, Chapel Hill, NC.

Acylonitrile (AN) is a potent carcinogen and a known rodent carcinogen. AN may undergo direct conjugation with glutathione or oxidation to form cyanhydrine oxides (CEO). CEO is metabolized to cyanide via several proposed pathways including epoxide hydration. Further, male mice were reported to be more sensitive to the acute toxicity of AN than females. The objectives of this work include the study of the importance of CYP2E1 and EH in the metabolism of AN to cyanide and the role of these enzymes in the gender differences in acute toxicity. As AN is a male wild-type (WT) and female wild-type (WT) and female CYP2E1-null mice received AN at 0, 2.5, 10, 20, or 40 mg/kg by gavage. Blood and tissue cyanide concentrations were measured at 1 and 3 hr after dosing. Present results showed that cyanide levels in blood and tissues of AN-treated WT mice were significantly greater than controls and increased in a dose-dependent manner. In contrast, cyanide levels in blood and tissues of AN-treated CYP2E1-null mice were not significantly different from those of vehicle-treated mice. Significantly higher levels of AN-derived cyanide were detected in the blood and tissues of WT male mice than in females, which may explain the higher sensitivity of male mice to AN-induced toxicity. Western blots of microsomal and cytosolic liver protein from WT male and female mice showed that while there was negligible differences in CYP2E1 expression, higher levels of EH were detected in male mice. Differences in the expression of EH may contribute to elevated metabolism of CEO to cyanide and to the greater sensitivity of male mice to AN-induced toxicity. In summary, these data demonstrated that CYP2E1 is the only enzyme responsible for the oxidative metabolism of AN and that metabolism of CEO via EH may play a major role in the formation of cyanide in mice. Further, gender differences in the expression of EH may explain the higher cyanide levels and the more severe toxicity observed in male vs. female mice.

610 DEHYDROEPANDROSTERONE (DHEA) TARGETS MULTIPLE NUCLEAR RECEPTORS TO MODULATE CYTOCHROME P450 GENE EXPRESSION.

S. L. Ripp, J. M. Peters, and R. A. Prough. 1Biochemistry, University of Louisville, Louisville, KY. 2Molecular Toxicology, Penn State University, University Park, PA.

DHEA is a C-19 sterol produced by the human adrenal gland. Administration of DHEA at pharmacological doses produces diverse biological effects, including chemoprotection in multiple cancer models, and amelioration of obesity, diabetes, and lupus in animal models and in humans. We have previously shown that in rain DHEA treatment modulates expression of multiple cytochrome P450 (CYP) en-zymes. DHEA treatment increases expression of members of the CYP4A and CYP3A subfamilies and decreases expression of CYP2C11. In the current study, we examined the role of nuclear receptors in modulation of CYP expression by DHEA. DHEA is known to activate a member of the nuclear receptor superfamily, peroxisome proliferator-activated receptor γ (PPARγ), to modulate expression of some genes, including CYP4A. PPARγ null mice were utilized to examine the role of this receptor in expression of CYP3A. In wild-type mice, DHEA-sulfate (150 μg/kg) induced CYP4A and CYP3A mRNAs by 4- and 1.5-fold, respectively, compared to controls. In PPARγ-null mice, induction of CYP4A by DHEA was lost while CYP3A induction was 1.5-fold, as in wild-type. Because CYP3A can be regulated by another nuclear receptor family member, the pregnane X receptor (PXR), we tested the ability of DHEA and its metabolites to activate PXR. A reporter gene containing two copies of the PXR responsive element from the CYP3A2 gene was transiently transfected into C-17 cells in the presence of absence of PXR expression vector. DHEA and its metabolites dione and androsterone were activators of PXR in these assays. We also examined the involvement of nuclear receptors in DHEA's ability to suppress CYP2C11. In cell-based assays, DHEA suppresses CYP2C11 in a PPARγ-independent manner. Taken together, these results indicate that DHEA can act through the nuclear receptors PPARγ and/ or PXR, and additionally members of the nuclear receptor superfAMILY to modulate expression of CYPs. Supported by D5K7477 (RNP) and F32 ES05927 (SLR).

611 CYTOCHROME P450 EXPRESSION DURING THE GASTRIC CARCINOGENIC PROCESS.


Gastric Cancer (GC) is the second most common cancer worldwide. In Mexico, gastric cancer has occupied the second and third places among neoplasms death rates for many years. GC is classified according to the histological type in intestinal and diffuse (Lai's Classification). It is known that intestinal GC is preceded by a series of histological changes that include active and inactive chronic gastritis, atrophy and intestinal metaplasia and finally gastric adenocarcinoma. Intestinal type GC is strongly related to environmental factors and the infection caused by Helicobacter pylori. The gastric tract is exposed to a large amount of carcinogens. It has been proved that 90% of all xenobiotics pass through it, most of them subject to be bioactivated by cytochrome P450 enzymes. Our main objective is to determine whether gastric cancer is the result of a CYP differential expression related to inflammation in the different histological progression stages leading to intestinal type GC. We sampled 244 gastric mucosal tissue specimens from the proposed gastric carcinogenesis model stages to be analyzed by immunohistochemistry performed with CYP2E1
612 CYP1B mRNA EXPRESSION IN TWO RELATED CATFISH SPECIES.

C. Metzer,1 R. T. D. Giulio,1 and K. L. Willett2. 1Department of Pharmacology, The University of Mississippi, University, MS 682; 2Nicholas School of the Environment, Duke University, Durham, NC.

CYP1B1 is a P450 gene that in mammals is involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs) and endrocholor to potentially toxic intermediates. This gene was recently cloned in fish (gup and plaice). The objective of our study is to characterize CYP1B1 in channel catfish (Ictalurus punctatus, CC) and brown bullhead (Ameiurus nebulosus, BB). These species were selected because of their established differences in sensitivity to PAH-induced carcinogenesis; BB is the more sensitive species. An 861 nucleotide sequence to the polyA tail was cloned from CC liver RNA. The complete cDNA sequence (104 of the 185 residues were shared by all species). A 459 nt sequence spanning two exons, there was only one amino acid residue different between the two catfish species. Tissue distribution was investigated in fish exposed to control and 20 mg/kg benzo(a)pyrene (BaP). RT-PCR analysis indicated a 207 nt CYP1B1 band in gill, blood, gonad, kidney, and liver of BB and CC. When standardized against beta-actin, there appeared to be an induction by BaP in some CC tissue but not liver where CYP1B1 was lowest. Complete Dose-Response curves for CYP1B1 mRNA induction by BaP and TCDD exposure of primary hepatocytes and gill cells are being established with real time quantitative PCR. Using semiquantitative RT-PCR, neither TCDD nor BaP caused significant induction in BB hepatocytes, however BaP caused some induction in CC hepatocytes. Ultimately, we will compare CYP1B1 induction responses in BB and CC to determine if differential CYP1B1 activity could have a role in species differences in PAH susceptibility.

613 ASSESSING THE ROLE OF CYP3A5 AND CYP3A7 IN DRUG-DRUG INTERACTIONS.


CYP3A5 is present in significant quantities in 20-60% of human livers, whereas CYP3A7 is more abundant in fetal liver. The role of these enzymes in drug interactions is not well understood. A panel of 16 compounds was tested for potential to inhibit dealkylation of 7-benzoxyl-4-trifluoromethylcoumarin (BFC) catalyzed by cDNA-expressed CYP3A5, CYP3A7, CYP3A4 and human liver microsomes (HLM). CYP3A4 was used as a non-CYP3A comparator. Three of 16 compounds exhibited 50% inhibition of CYP3A2 activity (IC50) whereas 10 compounds exhibited 50% inhibition of CYP3A4, CYP3A5, CYP3A7 and HLM activity. α-Naphthoflavone inhibited CYP1A2, but activated all CYP3A enzymes as well as HLM catalysis. The IC50 values for all CYP3A enzymes and HLM correlated well with each other (r > 0.77). We show that inhibitor specificity for CYP3A5 and CYP3A7 is similar to CYP3A4 but inhibitor potency for these enzymes is on average, 45-fold less than with CYP3A4. Since HLM is a mixture that includes at least CYP2A enzymes, we estimate the inhibitor potency when using HLM as an enzyme source in CYP3A4 inhibition assays. Indeed, this was observed. Because of similarities in inhibitor specificity between CYP3A4 and CYP3A7, CYP3A7-mediated drug-drug interactions and drug-endothelial interactions in the lung may exhibit a specificity profile similar to that of CYP3A4 in adults, however, inhibitor potency appears to be at least an order of magnitude less with CYP3A7.

614 DIESEL EXHAUST PARTICLE-INDUCED ALTERATIONS OF PULMONARY PHASE I AND PHASE II ENZYME SYSTEMS.

A. Rengasamy, M. W. Barger, F. Kan, J. K. Ma, V. Cattanach and J. Y. Ma. Pathology & Physiology Res. Br., NIOSH, Morgantown, WV.

Several environmental pollutants including polycyclic aromatic compounds induce carcinogenesis through the activation of xenobiotic metabolic pathways. The xenobiotics require bioactivation by phase I enzymes to induce carcinogenicity, while phase II enzymes contribute to resistance to chemical toxicity. The purpose of this study was to investigate the effects of diesel exhaust particle (DEP) on phase I and phase II enzymes. Male rats were intratracheally instilled with saline (vehicle control) or a single dose of Department or carbon black (CB) at 5, 15, or 35 mg/kg body weight. CB was used as a control for the particular carbon core of the DEP. At 1, 3, or 7 days post-exposure, lung microsomes and cytosol were prepared. The activities of CYP1A1 and CYP2B1 (Phase I enzymes) in microsomes and GST and catalase (Phase II enzymes) in the cytosol were determined. Enzyme protein levels were determined by Western blot analysis. Department exposure at 5, 15, or 35 mg/kg, nor CB, significantly elevated CYP1A1 protein levels at 1 and 3 days post-exposure compared to the control. CYP1A1 activity was also increased with 15 and 35 mg/kg DEP at 1 day post-exposure. On the other hand, both Department and CB exposure decreased CYP2B1 protein levels at 15 and 35 mg/kg with a concomitant attenuation of CYP2B1 enzyme activity. At 1 day post-exposure, both Department and CB significantly decreased the GST-P1 protein level at all doses tested with a significant attenuation in GST activity at 15 and 35 mg/kg. Catalase activity was significantly decreased by Department or CB (35 mg/kg) at 1 and 7 days. These results suggest that the organic components of Department induce CYP1A1, while the carbon core attenuates CYP2B1, GST and catalase activities. The Department-induced alterations in the phase I and phase II enzyme pathways may play a significant role in pulmonary toxicity/carcinogenicity.

615 METABOLISM AND TOXICITY OF THE FUNGICIDE THIRAM AND ITS METABOLITES IN RATS.

P. S. Dalvi, T. Wilder-Kofie, B. Mares, R. R. Dalvi and L. H. Billups. Biomedical Sciences, College of Veterinary Medicine, N. Idaho, Rexburg, ID.

Thiram (dimethyldithiophosphoric acid) is a diatoxidio compound widely used as an agricultural fungicide, antioxidant in rubber/linseed oil and antibacterial agent in medicine. The objective of this study was to examine whether thiram and its two metabolites (dimethylthiocarbamate (DMDC) and dithiocarbamic acid, interact with hepatic cytochrome P450 (CYP) CYP2B1 induced hepatic damage in rats. Thiram was administered at 600 mg/kg p.o. to rats at two equimolar doses (1.0 and 0.5 mmol/kg) and the animals were sacrificed at 3 and 24 hr after treatment. At 3 hr, there was a significant inhibition of CYP1A1 caused by only the higher dose of thiram and DMDC whereas the inhibitory effect was seen with both doses of carbon disulfide. On the other hand, CYP2B1 was inhibited by all doses of all compounds. CYP2B1 was not affected by DMDC, but was inhibited by higher dose of thiram and both doses of carbon disulfide. The activity of CYP2A6 was decreased by only the higher dose of carbon disulfide and not by others. The results of 24 hr treatment indicated that CYP1A1 and CYP2B1 were inhibited only by higher dose of thiram, CYP2B1 and CYP2E1 inhibited by higher dose of carbon disulfide and CYP3A2 remained unaffected by all doses of all three compounds. The higher dose of thiram elevated the levels of serum SDH and ALT at 24 hr. Further assessment of liver damage was done histopathologically, and only the higher dose of thiram produced mild to moderate hepatic cell necrosis 24 hr after treatment. In summary, the results suggest that these compounds are metabolized by CYP 1A1, 2B1 and 2E1, but not by 3A2, and only thiram is hepatotoxic. Supported by NIH/MBRS S06GM08091.

616 INCREASED CYP2E1 ACTIVITY AND ENHANCED SYSTEMIC OXIDATIVE STRESS AFTER ENDOTOXIN IN HUMANS.

R. T. Toivola, P. Van Eet, C. R. Cunningham, R. A. Bleisn and S. I. Sheidoff. 1 Medicine, VA Hospital, Lexington, KY; 2CDC, Unit of KY, Lexington, KY; 3College of Pharmacy, Unit of KY, Lexington, KY.

Reactive oxygen species (ROS) are important toxic mediators in the pathophysiology of systemic inflammation. Previous studies have shown systemic oxidative stress in animal models of inflammation. Usually activities of hepatic P450s are decreased. However, we previously reported that CYP2E1, a P450 that lacks ROS, is not decreased in humans after LPS. To further evaluate CYP2E1 and oxidative stress in the human LPS model, human volunteers were given LPS. Methods: Six healthy, non-smoking men were given two consecutive daily injections of E. coli lipopolysaccharide (LPS) as a safe reproducible model of inflammation. In a control study each subject was monitored after saline injection. Oral and formation clearing of chloroxacinone (CSX) as a measure of hepatic activity of CYP2E1 were assessed. Urinary F2-isoprostanes, which are derived from the lipids and cyclooxygenase NF-κB activation, and plasma nitrate/nitrite concentration were measured as markers of systemic oxidative stress, as well as plasma TGF-β1 and hyaluronic acid concentration. Results: Pharmacokinetic data of CSX clearance showed increases in the activities of CYP2E1 after LPS administration. Statistically significant two to three fold increases in urinary F2-isoprostanes were found between 0-6 hr after each dose of LPS. Plasma nitrate/nitrite concentration reached peak values of 86.0 ± 36μM and 70.8 ± 18μM between 3 and 6 hr after first and second LPS dose respectively, while the control value was 40.1 ± 18μM. The profiles of changes in leukocyte NF-κB (p50 and p65), human plasma TGF-β1 and plasma

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hyaluronic acid concentrations at early time points after each LPS administration exhibited transient but significant increases that returned to normal values within 24 hr after treatment. Conclusion: Our results indicate an increase in hepatic CYP2E1 activity with low-dose LPS in humans that is associated with enhanced but transient measures of systemic oxidative stress.

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PHENYLETHYLISOTHIOCYANATE (PEITC) REDUCES 2-AZINO-1-METHYL-6-PHENYLMDAzo[4,5- S]BIPYRIDINE (PHB) DNA ADDUCT FORMATION BY INHIBITION OF CYTOCHROME P450 DEPENDENT METABOLISM.

I.A. West, K. H. Dingley and K. W. Turnbaugh. Biology and Biotechnology Program, Lawrence Livermore National Laboratory, Livermore, CA.

Exposure to potent heterocyclic amine mutagens, such as PHB, result in DNA adducts that correlate with tumor formation in target tissues of rats. Consumption of cruciferous vegetables, which contain PEITC, appear to reduce the carcinogenic potential of heterocyclic amines. The mechanism by which PEITC reduces the carcinogenic potential is unknown. To determine if chemopreventives interfere with PHB bioactivation, microsomes from rat liver, prostate and lung were prepared to measure the metabolism of PHB by the presence of PEITC. In a separate experiment, rats were administered PEITC alone or in a PHB, exposure and DNA adducts were measured by acrylamide mass spectrometry. Results: Microsomes prepared from neither rat prostate, nor lung tissue generated oxidation products of PHB. In contrast, liver microsomes generated multiple oxidation products of PHB from including NOH-PHB, an intermediate that results in DNA binding. PHB metabolism in liver was inhibited by PEITC. With particular significance PEITC abolished the production of NOH-PHB, while less reactive metabolites were still produced. When rats were administered PEITC prior to PHB exposure, DNA adducts were reduced. We conclude: 1) NOH-PHB is not generated by cytochrome P450 at detectable levels in extra-hepatic tissue 2) PEITC inhibits cytochrome P450-dependent metabolism of PHB, and 3) inhibition the metabolic activation of PHB to reactive intermediate such as NOH-PHB leads to the reduction of DNA Adducts. This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. Research supported by USDOH USAMRDC # 45559PFD

USE OF REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (QRT-PCR) METHODOLOGY TO STUDY THE INDUCTION OF CYTOCHROME P450 (CYP) mRNA LEVELS IN RAT LIVER.


In this study QRT-PCR (TaqMan®) methodology has been employed to study the induction of CYP mRNA levels in rat liver and in cultured precision-cut liver slices. Fluorogenic (TaqMan®) probe-primer combinations specific for a range of CYP cDNAs were designed using Primer Express® software. The probe/primer sets were selected to span exon junctions in order to minimise the effect of any contamination from genomic DNA. Probes for CYP genes utilized a FAM reporter while the house-keeping (albumin) probe was labelled with VIC allowing duplex analysis for both CYP and albumin in the same reaction. To test the specificity of the TaqMan® probes and primers male Sprague-Dawley rats were treated with either a single 500 mg/kg ip dose of Arlocor 1254 (ARO) or four daily oral doses of 50 and 75 mg/kg/day deoxymethylen (DEX) and methylselenenapate (MCP), respectively. Total RNA was extracted from liver samples from control rats and pooled samples from ARO, DEX and MCP treated rats and cdNA prepared. A series of duplex PCR amplifications was conducted using an ABI PRISM™ 7700 Sequence Detector. Compared to albumin (as a house-keeping gene) levels of CYP1A1, CYP1A2, CYP2A1, CYP2B1 and CYP2D1 mRNA were induced by 51, 44, 49, 13 and 62 fold, respectively. To assess CYP mRNA induction in vitro, rat liver slices (diameter 10 mm, thickness around 250 μm) were prepared using a Krumdiek tissue slicer. Liver slices were cultured in Williams medium E containing 0.1 ml insulin, 0.1 mM DEX, 2 mM L-glutamine, 50 μg/ml gentamicin and 2.5 μg/ml funginone in a dynamic organ culture system at 37°C under an atmosphere of 95% O2/5% CO2. Treatment with ARO but not MCP for 24 hr markedly induced levels of CYP1A1 and CYP1A2 mRNA, whereas MCP induced levels of CYP4A1 mRNA. These results demonstrate the usefulness of TaqMan® QRT-PCR methodology for studying the induction of rat CYP mRNA levels in vivo and in vitro.

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MEASUREMENT OF HEPATIC ENZYME INDUCTION AND B-OXIDATION IN RATS DOSED WITH CLOFIBRATE.

D. E. Adamir and S. E. Boldt. Molecular and Investigative Toxicology, Croton Laboratories, Fiercer Global Research and Development, Croton, NY.

The goals of this study were to evaluate the effects of various doses of clofibrate on inducible enzyme in the rodent and to assess possible increases in peroxisome proliferation at the higher doses. Groups of male and female (Crl:CD®:CD) rats received either 60, 100, 450 or 900 mg/kg/day clofibrate i.d. for 14 days. The animals were sacrificed, and the livers removed and frozen. Subsequently the livers were homogenized, and supernatants were prepared and later assayed for peroxisome proliferation based on cytochrome B-oxidation of palmitoyl CoA. In comparison with control animals, palmitoyl CoA oxidation increased significantly (p < 0.01) in both sexes at 300 mg/kg, but appeared unaffected at 300 mg/kg clofibrate. A 100 mg/kg dose of clofibrate was found to be capable of generating an optimal response in the rat. Concurrently, liver microsomal samples were prepared from the same livers and later assayed for microsomal cytochrome P450, NADPH cytochrome c reductase, cholesteryneurin O-dealkalase (EROD), methoxyresorufin O-dealkalase (MROD), pentoxyresorufin O-dealkalase (PRD), and nitrosemphine N-demethylase (EEM) activities. For some of these endpoints, significant (p < 0.05) differences were found between the control means and those means obtained from animals dosed with clofibrate. In both sexes, significant elevations in PRD and NOD were seen at all three dose levels. Elevated cytochrome P450 reductase was noted in both sexes at the high dose. Both EROD and MROD were essentially unaffected in males and females. In males, elevations of total P450 were observed at 300 and 450 mg/kg. In females, EMD levels rose significantly at all three doses, and increases were also seen in NP at 300 and 450 mg/kg. Based upon results for several inducible rodent cytochromes, this study demonstrates that clofibrate is a hepatic microsomal enzyme inducer of CYP2B1 and/or CYP2E1 in both sexes and CYP3A1 in females.

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IRON DEFICIENCY CAUSES MANGANESE ACCUMULATION IN DEVELOPING RAT BRAINS.

K. M. Erikson and M. Aschner. Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC.

Iron (Fe) deficiency (ID) is a prevalent nutritional disorder affecting nearly 2 billion people worldwide. It is well known that ID represents a risk factor for toxicity due to metal exposure (e.g., aluminum, cadmium, lead). Since Fe and manganese (Mn) share similar transport mechanism(s), studies were carried out to test the hypothesis that dietary ID leads to increased Mn accumulation in the brain, potentially predisposing the central nervous system (CNS) to toxicity. Previous studies have shown that ID increases brain Mn, but specific regional changes have, as of yet, not been reported. Thus, the goal of this study was to examine the effect of ID on Mn in Mn-rich brain regions. Twenty-one day old male Sprague-Dawley rats were fed for 12 weeks one of three semi-purified diets (AIN-93G, 30 mg Fe/Kg, 10 mg Mn/Kg diet, CN n = 7), (3 mg Fe/Kg, 10 mg Mn/Kg diet, ID n = 7) and (3 mg Fe/Kg, 100 mg Mn/Kg diet, IDn n = 7). After 6 weeks of dietary treatment, the rats were euthanized, and the brains dissected into seven brain regions. Brain regions were analysed for [Mn] using neutron activation analysis (NAA), and amino acid concentrations (glutamate, aspartate, glutamine, glycine, taurine, GABA) concentrations were measured with HPLC. Both Fe-deficient diets caused significant (p<0.05) increases in [Mn] in six of the brain regions compared to controls (ranging from cerebellum, 30% globus pallidus 60% increase). The hippocampus was the only brain region in which the IDn+ group accumulated significantly more Mn than both the CN and ID groups (CN<1<CN<1+1<IDn). While multiple alterations in brain regional amino acid concentrations were observed, two significantly correlated with CNS Mn. In the substantia nigra, Mn significantly correlated with increased taurine concentrations (r=0.65; p<0.05), whereas in caudate putamen [Mn] negatively correlated with decreased d-amino butyric acid (GABA) (r=-0.54; p<0.05). These data show that ID increases brain Mn in a heterogeneous fashion, and that ID is a significant risk factor for CNS Mn accumulation. (Supported by NIEHS 10563).

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PRENATAL LEAD (Pb) EXPOSURE AND SCHIZOPHRENIA: METHODOLOGY FOR EXAMINING THE POTENTIAL RELATIONSHIP.


Schizophrenia is a mental disorder affecting approximately 2.8 million Americans, characterized by hallucinations, delusional and social withdrawal, and disorganized thinking. The causes of the disease are thought to include hitherto unspecified genetic and environmental risk factors. Prior studies suggest that disruptions in prenatal development may be risk factors. The current study was designed...
to test the hypothesis that exposure to a specific neurodevelopmental disruptor (Pb) during the 2nd trimester of pregnancy might increase the risk of developing the disease. Serum samples from a cohort of 19, 044 pregnancies were collected between 1959-1966. Samples of serum were taken at each trimester and at delivery, respectively. Samples were stored at 20°C until the present day for an average storage time of 38 years. Diagnosis of schizophrenia spectrum disorder in the children born to this cohort yielded a total of 71 cases. A method to quantify a biological marker of Pb exposure, i.e., delta-aminolevulinic acid (d-ALA) has been developed. Serum samples were processed and treated with acetylaceetone and derivatized with formaldehyde to produce a fluorescence readout. The samples were analyzed on a reverse phase C18 column and assayed by a fluorescence detector with an excitation wavelength set at 360 nm and an emission wavelength of 470 nm. Our method improves the detection limit by a factor of 10, ultimately yielding 2.4 ng/mL of d-ALA. d-ALA values greater than (and less than) 10 ng/mL agree well with PbB values greater than (and less than) 14 μg/dL. This work is currently under review on the primary study.

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COGNITIVE EFFECTS IN LEAD EXPOSED CHILDREN FROM URBAN AREAS IN THE REGION LAGUNERA, MEXICO.

G. G. García-Vargas1, M. Rubio-Andrade1, E. Casillas1, E. Vera-Aguilar2 and M. E. Cebrían1.1. Medicina, UJED, Gómez Palacio, Dgo, Mexico. 2. Psicología, UJED, Gómez Palacio, Dgo., Mexico, 3. CINVESTAV-IUNAM, Mexico DF, Mexico. 4. Medicina, UA de C. Torreón, Coah., Mexico.

There is known that exposure to low doses of lead can cause cognitive effects in children. This population study was based in a previous cross sectional survey, where it was demonstrated that heavy metal contamination in soil, dust, and air around a smelter site by exceed backround levels, and that a high proportion (> 90%) of children living in the neighborhood had lead in blood concentrations (PbB) above 10 μg/dL (J. Toxicol. Environment Health. Part A, 62, 417-429, 2001). From initial number of 394 scholar children, 285 were recruited and their parents filled a consent letter form, to be studied by psychometric tests, particularly the Wechsler Intelligence Scale for Children - Revised (WISC-R) for Mexican children in Spanish. Diet, socioeconomic status, hygienic habits, and other variables were assessed by questionnaire. Gender distribution was 151 males and 134 female, aged 6 - 14 years. The mean (range) of PbB was 19.13 μg/dL (3.9 - 58.5 μg/dL). Same parameters for psychometric index were: IQ: 100.52 (75.0 - 134.0) and for equivalent mental age: 8.13 years (5.0 - 11.5 years). Non parametric Spearman’s correlation were significant between lead and IQ (p = 0.105% p = 0.004%) and for lead and equivalent mental age (p = 0.020% p = 0.0001). Socioeconomic status and education levels were co-variables for PbB and IQ, however, when the results were adjusted by the co-variables, the relationships among PbB and IQ, and equivalent mental age remain significant: The results confirm that chronic lead exposure have cognitive effects on children, particularly in mental maturity. These results will be useful for follow-up studies and set up epidemiological surveillance programs in this population.

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HEME BIOSYNTHESIS IMPAIRMENT IN CHILDREN CHRONICALLY EXPOSED TO LOW DOSES OF LEAD IN A SMOLET COMMUNITY IN MEXICO.

J. Ruvalca1, A. Cebrián1, P. López2, J. Alarcón1, E. Vera3, M. Uribé1, M. E. Cebrián1, J. L. Rosado2 and G. G. García-Vargas1. 1. FM-UJED, Gómez Palacio, Dgo., Mexico, 2. INNSZ, Mexico, DF, Mexico. 3. Psicología, UNAM, Mexico DF, Mexico. 4. CINVESTAV-IUNAM, Mexico, DF, Mexico, 5. FM-UA de C. Torreón, Coah., Mexico and 6. UAC, Queretaro, Qro, Mexico.

Metal contamination in soil, dust and air around a smelter site has resulted in increased lead body burden in children living in the vicinity of the major smelter complex in Latin America. Chihuahua, northern Mexico. Children with lead in blood concentrations (PbB) over 10 μg/dL (value considered as indicative of lead poisoning) are at risk of suffering adverse effects of lead. Heme biosynthesis can be disturbed by lead exposure. Several heme biosynthesis enzymes or intermediate metabolites has been used as biomarkers in lead toxicity. The aim of this work was to assess two of the main lead toxicity biomarkers related with heme synthesis, particularly we evaluate the activity of the 5-aminolevulinic dehydratase (ALA-D; assayed by HPLC) and zinc protoporphyrin (ZPP; measured by fluorescence) in 270 children aged from 6-7 years old. Children were recruited from nine primary schools, 2 km around the smelter. Diet, socioeconomic status, hygienic habits, and other variables were assessed by questionnaire. Median (range) of PbB in children was 11.18 μg/dL (3.0 - 43.82 μg/dL), whereas for ZPP was 65.0 mmol of ZPP per ml of Hb (27.0 - 452.0 mmol of ZPP per ml of Hb), and for ALA-D was 147.0 mmol ZPP / ml RBC x h (28.9 - 332.9 mmol ZPP / ml RBC x h). There was a significant inverse relationship (r = -0.467; p < 0.0001; equation: Log (y) = 0.869 [log x] + 3.167) between PbB and ALA-D, and a significant positive relationship between PbB and ZPP. These results remain significant even adjusted by hemoglobin. These results showed that heme biosynthesis biomarkers, particularly ALA-D activity and ZPP in blood are useful biomarkers of damage for lead toxicity in children exposed to low doses of the metal.

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LOW-LEVEL POSTWEANING LEAD (Pb) EXPOSURE: ALTERATIONS IN TIMING BEHAVIOR.

B. Brodeur, J. Coleman, D. Cooenfellt, T. Smith and C. Lubbers, Department of Psychology, Kansas State University, Manhattan, KS.

Several clinical and experimental studies have suggested that low-level lead (Pb) exposure impairs impulsive behavior and/or delay aversion. In addition, Pb exposure is associated with deficits in temporal behaviors, i.e. fixed-interval schedules. However, no studies have been conducted to determine if these Pb effects are related to differences in timing mechanisms. To test this hypothesis, rats exposed chronically from weaning to 10, 30, or 50 ppm Pb acetate in drinking water were tested on a temporal discrimination task. Rats were trained to respond on one lever after the presentation of a 4-s ("short") tone and on another lever after the presentation of a 16-s ("long") tone. Once all subjects reached a criterion of 80% correct for both the 4-s and 16-s tones, intermediate tones (5.2, 6.4, 8.0, 10.0, and 12.8 s) were added to the trials procedure. For each rat, the percentage of 4-s responses at each signal duration was calculated, and psychophysical functions were determined by using least squares fitting to the power law, defined as the stimulus duration at which a rat responds "long" 50% of the time and the difference limen, an estimate of the threshold to detect differences between two time lengths was calculated from the individual psychophysical functions. Pb exposure produced a decrease in the PI without altering the DL; Pb exposure had no effect on the ability to discriminate the 4 and 16-s sec. These results indicate that Pb produced an increase in clock speed without altering threshold sensitivity or discrimination ability and support the hypothesis that alterations in timing mechanisms play a role in some Pb-related behavioral deficits. Funded by NIEHS (ES01992).

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EFFECTS OF CHRONIC LEAD EXPOSURE ON HIPPOCAMPAL BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) CONCENTRATIONS IN RATS.

S. M. Lailey, Biomedical & Therapeutic Sciences, Univ. of Illinois College of Medicine, Peoria, IL.

Previous studies have established that chronic developmental lead (Pb) exposure impairs the magnitude and duration of long-term potentiation (LTP), the amount of stimulus-evoked hippocampal glutamate release. Other evidence indicates that induction of LTP is dependent on appropriate BDNF expression and release, and BDNF also potentiates glutamatergic transmission by increasing the efficacy of glutamate release. Moreover, chronic Pb exposure has been reported to decrease neurotrophic factor gene expression (Schneider et al., 2001). This study examined the production of hippocampal BDNF protein in rats chronically exposed from birth. Pregnant Long-Evans dams were exposed to 0.2% Pb in drinking water beginning at gestational day 16 and exposure was continued throughout lactation with male offspring continued on the same solution until testing. Previous studies have shown that this exposure protocol produces blood and brain Pb values of 40 μg/dL and 378 μg/g tissue, respectively (Gilbert et al., 1999). At 8 months of age rats were sacrificed and dorsal hippocampi and parietal cortices were dissected and sonicated. Cell lysates were clarified by centrifugation, briefly acidified in phosphate-buffered saline, and BDNF quantified by a microplate-based sandwich enzyme immunoassay; signal was determined by the absorbance at 450 nm. Total protein was determined by the biuretine method. Hippocampal expression of BDNF protein did not differ between Pb-exposed and control animals. While alterations in BDNF activity could conceivably be an important factor in the impaired LTP and diminished glutamate release observed in behaviorally-impaired or depolarized hippocampal tissue, these data suggest that such exposure-induced changes cannot be traced to alterations in the expression of BDNF protein in un-perturbed animals. (Supported by the Children's Miracle Network)

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TOXICOLOGICAL ASPECTS OF LEAD: COGNITIVE EFFECTS IN CHILDREN AND NEUROCHEMISTRY IN YOUNG ALBINOS.

M. Al-awady and W. Atta, Faculty of Medicine, Zagazig University, Zagazig, Egypt. Sponsor: L. Salata.

Although little is known about the mechanism of lead neurotoxicity, the hazardous effects of lead exposure on the young has long been recognized. The current study investigated the effects of lead exposure on the intelligence and cognitive abilities of
children, as well as on gamma amino butyric acid (GABA) levels in the brains of young albino rats. Three hundred children of both sexes selected from different residential areas, were investigated for their blood lead level ( BLL), IQ and mineralization state. The clinical study on the children revealed that there was a strong relationship between the place of residence and lead exposure. Children living in rural areas had the lowest blood lead levels, while those living in the urban areas had the higher blood levels. Children living in the rural areas had higher IQs and cognitive abilities, compared to those in urban areas. Fifty young albino rats divided into 3 equal groups were exposed to 0, 100 and 1000 ppm lead acetate in drinking water respectively for 4 weeks. BLL and GABA levels were determined at the end of 2nd, 4th, and 6th weeks. No behavioral changes were noticed upon exposure to the examined doses of lead. However, lead exposure at 100 and 1000 ppm was found to elevate GABA levels. At the end of the follow up period (the end of the 6th week), GABA levels decreased toward normal control levels at the end of the 6th week although BLL remained high.

627 DEVELOPMENTAL LEAD EXPOSURE AFFECTS NEUROCHEMICAL AND IMMUNOHISTOCHEMICAL MARKERS OF THE RAT DOPAMINERGIC AND SEROTONINERGIC SYSTEMS.


CEATOX, UNESP, Botucatu, Brasil and INTD, USEPA, Research Triangle Park, NC.

Recently, we have demonstrated that exposure to lead (Pb) during pregnancy and lactation induced behavioral alterations in rats on PN23 and on PN70. Considering the role that the dopaminergic and serotonergic systems play in behaviors, this study was undertaken to investigate the effects of Pb exposure on these neurotransmitter systems. Wistar rat dams received 500 ppm of Pb acetate or 660 ppm Na acetate in the drinking water during pregnancy and lactation and the pups were euthanized on PN23 or 70. Dopamine (DA) and its metabolites DOPAC and HVA, as well as serotonin (5-HT) and its metabolite 5-HIAA were determined (ng/g of tissue) in the hypothalamus, hippocampus and striatum. Cortical sections immunostained for tyrosine hydroxylase (TH) or 5-HT were used for morphometric analyses ( # of cells and volume) of the ventral tegmental area (VTA), substantia nigra (SN) and dorsal raphe nucleus (DRN). Data were analyzed by three-way ANOVA (Treatment x Age x Region). Data are presented as mean±SE. Means followed by different letters are statistically different. These results show that low level Pb exposure during pregnancy and lactation induce neurochemical and morphological alterations in a brain region-specific manner that may be related to the behavioral alterations previously observed.

629 ENVIRONMENTAL ENRICHMENT REVERSES COGNITIVE DEFICITS INDUCED BY LEAD.


Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.

Children exposed to lead (Pb2+) exhibit long-term deficits in cognitive function. A recent study has shown that chelation therapy, the primary means of treating Pb2+-poisoned children, has no significant impact on Pb2+-induced cognitive deficits (Rogat et al., N.Engl.J. Med., 344: 1421, 2001). Thus, finding alternative intervention strategies is imperative. We have previously described that rats exposed to Pb2+ during gestation and lactation until weaning, exhibit deficits in spatial learning as young adults (Kuhlman et al., Neurosci.Lett. 233: 101, 1997). Using this Pb2+ exposure protocol, we set out to determine if environmental enrichment (EE) affected the learning deficits induced by Pb2+. One male rat from control or Pb2+-exposed litters was tested at 21 days postnatal on the water maze test. Rats were reared in standard housing conditions from postnatal day 21 (weaning) until the end of the water-maze spatial learning task that started at postnatal day 50. Lead-exposed rats reared in EE acquired the spatial learning task to the same degree as control EE rats. On the other hand, Pb2+-exposed rats reared in standard housing conditions were markedly impaired in the acquisition of the spatial learning task. Probe test data confirmed that Pb2+-exposed rats housed in an enriched environment were able to learn the task while those in standard housing did not. No group differences were noted for body weight, swimming speed or cue use (non-spatial). These findings demonstrate for the first time that EE initiated after developmental exposure to Pb2+ is able to totally reverse the spatial learning deficits induced by Pb2+. In summary, our studies suggest a new direction for the treatment of children with learning disabilities resulting from environmental Pb2+ exposure. [Supported by grant # ES06189 to TRG].

630 ALTERED NEUROGENESIS IN THE DENTATE GYRUS OF LEAD-EXPOSED RATS: PRELIMINARY FINDINGS.


Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.

Chronic exposure to lead (Pb2+) during development diminishes spatial learning in young adult rats (Nish et al., Neuroscience 99: 235, 2000). Recent studies suggest that modulation of neurogenesis in the dentate gyrus (DG) can influence the ability to learn a spatial learning task. To investigate whether Pb2+-induced spatial learning deficits are associated with altered neurogenesis in the DG, we examined cell proliferation in rats chronically exposed to Pb2+ using the thymidine analog bromodeoxyuridine (BrdU). Rats were injected with BrdU (200 mg/kg or 100 mg/kg daily) and perfused transcardially (two hours or five days, respectively) at postnatal day 50. BrdU labeled cells were counted in the suprapyramidal blade (SPB), infrapyramidal blade, dentate crest, and hilus. BrdU-labeled cells localized in the subgranular zone (SGZ) of the DG were irregular in shape and frequently arranged in clusters. Within the granular cell layer, labeled cells were seen occasionally and only after five days of BrdU injection. In rats perfused two hours after BrdU injection, cell counts showed no significant difference in any DG region as a function of treatment. On the other hand, significant treatment effects on the number of BrdU-labeled cells were found in animals injected for five days. Pb2+-exposed rats exhibited a lower number of BrdU labeled cells in the SGZ of the suprapyramidal blade (24.3%, p < 0.05) and in the SGZ and granule cell layer of the suprapyramidal blade compared (21.8%, p < 0.05). Total number of BrdU labeled cells in the entire SGZ were also decreased, but the difference did not reach a statistical significance (p = 0.091). These observations suggest that chronic developmental exposure to Pb2+ decreases net neurogenesis, possibly by reducing the survival of proliferating cells in specific areas of the DG. This alteration in DG neurogenesis may reflect a novel mechanism by which developmental exposure to Pb2+ causes deficits in spatial learning.
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GESTATIONAL EXPOSURE TO MERCURY RETARDS ORGANIC BEHAVIOR IN TRANSITION IN ADULT RATS.

M. C. Newland, P. A. Reille, J. L. Langston and E. M. Palez. Psychology, Auburn University, Auburn, AL.

Evidence is growing that developmental exposure to methylmercury (MeHg) has behavioral effects that extend into adulthood and aging. To study MeHg's prolonged effects on behavioral plasticity, rats were exposed to methylmercury during gestation via maternal drinking water containing 0.05 or 6.4 ppm Hg as MeHg. Exposure began at least four weeks before mating, and continued to post-natal day 16. Brain Hg concentrations were 0.49 and 9.8 ppm at birth in littersmates of the rats described here; lactation resulted in no mercury exposure. The behavior of one- and two-year old offspring was maintained under various Concurrent Variable Interval reinforcement schedules in which pressing each of two levers was maintained by independent Variable Interval schedules of reinforcement, and t is the average inter-reinforcer interval. During the first 30 minutes of 150 min. sessions, the reinforcement rates on both levers were equal, and the ratio of left to right (L/R) lever presses approximated 1.0. After 30 min. the left lever became rich relative to the right, the right became relatively rich, or there was no change. During sessions in which one lever became rich, responding was tracked as it transitioned to reflect the new allocation of reinforcements. Terminal reinforcement ratios (L/R) used were 9:1, 4:1, 3:1, 1:1, 1:3, 1:4, and 1:9. In controls and most exposed rats, response ratios reflected reinforcer ratios by the end of the session. The transitions of many exposed rats were retarded relative to controls, and in some transitions they went in the wrong direction. The tactic used here greatly reduced the time required to study behavior in transition from a month in previous reports to a single session here. (Supported by ES04646 from NIEHS).

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GESTATIONAL EXPOSURE TO CHLORPYRIFOS: QUALITATIVE AND QUANTITATIVE NEUROPATHOLOGICAL CHANGES IN THE FETAL NEOCORTEX.

L. L. Laster, D. W. White, S. Padilla and S. Barone. Toxicology, University of North Carolina, Chapel Hill, NC and Neurotoxicology, USEPA, Research Triangle Park, NC.

This study investigated the qualitative and quantitative neuropathological changes that occur in the fetal brain following gestational exposure to chlorpyrifos (O-Diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothionate), a commonly used organophosphorus insecticide. Two cohorts of pregnant rats were orally dosed daily with chlorpyrifos in corn oil on gestational days 14-18. Cohort A consisted of a time course study of fetuses collected at 2, 5, 10, and 24 hours after the last dose of chlorpyrifos (0 or 7 mg/kg/day). Cohort B is a replication of Cohort A and includes a Dose-Response component at 5 hours (0, 1, 3, 5, and 7 mg/kg/day of chlorpyrifos). Fetal brain total cholinesterase, acetycholinesterase, or butyrylcholinesterase activities were 70, 75, or 53 % of control activity 5 hours after the last 7 mg/kg dose of chlorpyrifos. Fetal brain sections stained with cresyl violet for neuropathological assessments showed no pathology in the neocortex, consisting of vascular alterations, disorganized migratory waves, ectopic cells, neuropathological holes, and qualitative thinning of the ventricular zone and cortical plate. Neuropathological changes were evident at dosages as low as 1 mg/kg/day. Stereology was used to quantify the volume of the ventricular zone, subventricular zone, and cortical plate in the fetal neocortex showed limited treatment-related changes in the volume of the subventricular zone 7 mg/kg/day but no change in other neuropathological markers. We conclude that repeated, gestational exposure to chlorpyrifos does not inhibit cholinesterase activity in the fetal brain, but also causes neuropathological changes in the fetal neocortex. This abstract does not reflect EPA policy.

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PRENATAL EXPOSURE TO CHLORPYRIFOS ALTERS NEUROTROPHIN IMMUNOREACTIVITY AND APOPTOSIS IN RAT BRAIN.


The effects of the organophosphate pesticide chlorpyrifos (CPF) on the regional distribution of three neurotrophic factors that regulate brain development and on levels of apoptosis. Pregnant Long-Evans rats were orally dosed daily (p.o.) with CPF (0, 3, 5, 7, and 10 mg/kg) in corn oil on gestational days 14-18. Fetuses were examined at 2, 5, 10, and 24 hr after the last dose. Immunohistochemistry (IH2) showed very little nerve growth factor (NGF) immunoreactivity (IR) in the fetal brain. There was significant brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) IR in the ventricular and subventricular zones fetal brains. BDNF IR was widely and spatially distributed throughout the intermediate zone and cortical plate of the control neocortex. There was a decrease in BDNF and NT-3 IR in the brains of CPF treated fetuses. These CPF-related changes in NT-3 IR are consistent with quantitative changes in whole brain BDNF and NT-3 levels determined by enzyme-linked immunosorbent assays (ELISA). The IHC demonstrates that these changes are limited primarily to proliferative zones of the forebrain. Results from cell death ELISA showed the highest level of DNA fragmentation at the 7 mg/kg/day dose at 5 hr after the last dose. Terminal transferase DUTP nick end-labeling (TUNEL) assays of fetal brains showed increased numbers of apoptotic cells in the striatum, hippocampus and cortical plate. This apparent increase in apoptosis was localized primarily to proliferative zones in the striatum and the hippocampus. The altered pattern of apoptosis in the fetal brains of CPF-exposed rats were reciprocal to changes in BDNF and NT-3 IR in proliferative and post migration zones. This observed increase in apoptosis could be due to the changes in neurotrophin levels. Further, these changes in regional levels of neurotrophins and apoptosis with CPF exposure suggest possible alterations in cell number and function that could persist in the adult nervous system. This abstract does not reflect EPA policy.

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CHLORPYRIFOS ALTERS GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IN THE DEVELOPING RAT BRAIN: DISRUPTION OF ASTROCYTE DEVELOPMENT?

S. L. Garcia, E. L. Seidler and T. A. Slukin. Pharmacology and Cancer Biology, Duke University, Durham, NC.

Despite limitations recently imposed on the organophosphorus pesticide, chlorpyrifos (CPF), concerns remain regarding its potential neurotoxicological properties in the developing nervous system. CPF inhibits brain cell replication and prevents differentiation but the specific types of cells targeted by CPF have not yet been identified. CPF affects glial cells in culture. We therefore administered CPF to developing rats and examined expression of the astrocytic protein, GFAP, using immunoblotting. Prenatal treatment increased GFAP levels in fetal brain only at CPF exposures that elicited maternal toxicity (reduced weight gain), and no persistent changes were seen at low postnatal day (PN) 30. CPF treatment on PN1-4, at doses below the threshold for systemic toxicity (1 mg/kg/day) elicited initial suppression and rebound elevation of GFAP, limited to the cerebellum of males. Bergmann glia, responsible for guiding neuronal migration, are emerging in the cerebellum at that time, and our results may thus explain the targeting of motor function in males with this treatment regimen. When we revisited CPF later, during the peak of gliogenesis (PN11-14, 5 mg/kg/day), GFAP showed initial deficits across all brain regions and in both sexes; rebound elevations (PN30) were seen in all regions in males, with the largest effect in striatum (significant for both males and females). Our results indicate that CPF targets glial development in vivo, so that the postnatal period is a critical period of susceptibility. The region-, sex-, and time-dependent effects on astrogliosis are likely to contribute to comparably selective alterations in synaptogenesis, axonogenesis, and ultimately, behavior. Glial targeting suggests that the vulnerable period for CPF neurotoxicity extends relatively late in brain development, into the phases of synaptic plasticity, myelination, and architectural modeling. (Supported by USPHS ES10396, ES10387, and an EPA STAR fellowship)

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DEVELOPMENTAL NEUROTOXICITY OF CHLORPYRIFOS: DEFINING THE VULNERABLE PERIOD.


There is concern over the consequences of fetal and childhood exposure to chlorpyrifos (CPF) and related pesticides. In previous work, we found that maternal CPF produced cell damage/loss in the brain, with abnormalities of neurotransmission and attendant behavioral anomalies. We used the same biomarkers to examine prenatal treatment "windows" to define the critical periods in which neural and synaptic development show immediate effects of CPF. One group of pregnant rats received 0, 1, 2 or 5 mg/kg/day by sc injection from gestational days (G) 9-12 during neural tube formation. A second group received 0, 1, 2, 5, 10, 20, or 40 mg/kg daily on G17-20, a peak period of neurogenesis. In the G9-12 treatment group, the threshold for maternal toxicity, defined by reduced weight gain, was 5 mg/kg; however, even this was only a transient reduction. There was no reduction in the number of fetuses assessed on G17 or G21, nor were there significant changes in fetal weight, brain weight, heart weight or liver weight. When the treatment window was shifted to G17-20, the threshold for impairing maternal weight gain was slightly higher (10 mg/kg), and although a similar threshold was seen for impairment of fetal body and liver weights, both the heart and brain were spared. DNA content, a marker for cell number, was unaffected in brain regions, although reductions were seen in the fetal liver at doses exceeding the threshold for maternal toxicity. These results stand in contrast to the robust effects seen previously for im
mediate effects of postnatal CPF treatment on brain development, where apparently subtoxic doses nevertheless elicited reductions in brain cell number. It remains possible that doses from prenatal CPF exposure may emerge later in development. We have preliminary evidence that behavioral anomalies emerge in adolescence or young adulthood. However, in terms of immediate biomarkers of neuronal damage, it appears that later stages of development are more sensitive. (Supported by USPHS ES10387 and ES10356, and by the Leon Golberg Toxicology Fellowship)

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NEUROCHEMICAL EFFECTS OF REPEATED DEVELOPMENTAL EXPOSURES TO CHLORPYRIFOS ON THE CHOLINERGIC NERVOUS SYSTEM

J. R. Richardson and J. E. Chambers. Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

Neurochemical components of the cholinergic system play critical roles in the development of the nervous system. The widely used organophosphorus insecticide chlorpyrifos (CPF) has been shown to affect the levels or activities of many of these components. The effects of repeated exposures to CPF during the gestational and postnatal periods on the activities of brain cholinesterase (CHE) and choline acetyltransferase (ChAT) were studied. Pregnant Sprague-Dawley rats were orally administered CPF daily from gestation days 6-20 at doses of 0 (control), 5, or 50 mg/kg/day. Following parturition, designated as postnatal day (PND) 0, pups were orally gavaged with CPF from PND1-21 at doses of 0 (control), 5, or 50 mg/kg/day. CHE activities increased from 3.5 and 11-fold from PND6 to PND30, respectively. CHE was inhibited by about 26% on PND1 in the SC group and had returned to control values by PND6. CHE was inhibited in a dose-related manner in all treatment groups, with inhibition of about 22 and 24% on PND6 in the CL and 5L groups, respectively, and on PND12 by about 23, 51, 31, and 47% in adult and juvenile CL, CH, SL, and 5H groups, respectively. CHE inhibition on PND22 was about 12, 52, 16, and 52% in the CL, CH, SL, and 5H groups, respectively, and persisted in the adult and juvenile groups over the period of CPF exposure. In adult rats, CHE activity was decreased by 11% only in the 5H group on PND22 and 30. These data suggest that exposure to CPF during the gestational and postnatal periods results in a relatively persistent inhibition of CHE. In addition, exposure to CPF during the gestational period may have resulted in an increased susceptibility of these animals to decreases in ChAT activity elicited by subsequent postnatal exposures to low levels of CPF. (Partially supported by NSF EPS-98784698)

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BEHAVIORAL EFFECTS IN TWO GENERATIONS OF RATS FOLLOWING DAILY PRE/POSTNATAL EXPOSURE TO TECHNICAL CHLORIDAN

V. V. St. Omer, K. M. Muzinda, B. C. Danzi, E. O. Abadla, M. M. Mankwane, F. Johnson and R. D. Moshupa. Biomedical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA. All others, Veterinary Medicine, University of Pretoria, Pretoria, South Africa. Sponsor: R. Danzi.

Male and female Sprague-Dawley rats were used in a two-generation study to evaluate neurobehavioral toxicities of technical grade chloridan, a mammalian toxicant and a nonpersistent pesticide. Adult and juvenile rats (F0, F1, F2) were exposed in replicate-1 to continuous levels of chloridan (0.0, 0.125, 0.25 and 1.0 mg/kg) by oral gavage. Offspring (F1 and F2) were exposed to the same levels in utero and at lactation until weaning. In the F1 generation, chloridan at 1 mg/kg body weight (bw) delayed (p < 0.05) and female negative geotaxis (PND 7-10), and olfactory discrimination (PND 11), but advanced both surface righting reflexes (PND 3-4) and spatial learning abilities in Morris maze (PND 77); at 0.25 mg/kg chloridan advanced female Morris maze spatial learning. For F2 generation, chloridan at 1 mg/kg bw delayed female olfactory discrimination and fore-limb grip strength; at 0.25 mg/kg chloridan delayed male and female surface righting reflexes and male hind-limb grip strength. In general, chloridan manifested greater neurobehavioral changes in the F1 generation compared to F2. The female rats were more sensitive than the males to chloridan effects (Supported by MHPF/AIT/SDR # 505/0/1992256008008)

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REPRODUCIBILITY OF DEVELOPMENTAL NEUROTOXICITY PRODUCED BY PHYRROIDTS AND DDT IN NEONATAL MICE.


Eriksson et al. (1) found that mice showed persistent changes in neurochemistry and behaviour after administration of very low doses of pesticides such as phyrroidts or DDT to the neonate. We examined the reproducibility of these effects. Three trials of a single dose of DDT (1 mg/kg) to 10 day old mouse pups produced small but significant decreases (p=0.01 or <0.001) in neocortical muscarinic receptor density at 4 months, which was absent at 0.5, 5 and 100 mg/kg, and re- sembled that reported by Eriksson et al. (1). After daily administration of phyrroidts (bioalcohol or deltamethrin) (0.7 or 3.5 mg/kg) from the 10th to the 16th postnatal day, neocortical muscarinic receptor binding at 4 months showed no consistent changes. Behavioural tests at 4 months showed significantly delayed habituation of face field behaviour in males and females, but 1st trial at 0 mg/kg and DDT dose level. Neither phyrroidts produced a change in habituation. Although our baseline values for muscarinic receptor density (813-1321 fmol/mg protein) were similar to those reported by Eriksson et al. (1), we could not reproduce the rapid habituation shown by their control mice, despite the use of the same strain and similar test conditions. We can confirm the reported developmental effects of DDT on muscarinic receptors but found that those of phyrroidts were not reproducible. Reported behavioural effects were also not reproducible, although the latter may be due to baseline differences. We conclude that although neonatal exposure to DDT produces reproducible and lasting changes in neurochemistry, phyrroidts do not produce a robust effect, possibly due to their rapid disposal from the body or to a dependence on as yet undefined variables. (1) Eriksson R, Albison J & Fredrickson A (1992 Brain Res. 582, 277-281)

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EFFECTS OF DEVELOPMENTAL EXPOSURE TO HEPHTACHLOR ON THE CHOLINERGIC SYSTEM IN RATS.

S. Parkison-Parker, K. L. McDaniel and V. C. Moser. NTDI/NEUBORAD, USDA, Research Triangle Park, NC and 'Curriculum in Toxicology, UNC, Chapel Hill, NC.

Hephtachlor is an environmentally persistent cyclodiene pesticide which is a known antagonist of the 5-hydroxytryptamine-4 (5-HT3) receptor. Since 5-HT3 is a tropic factor for the development of other neurotransmitter systems (Lauder et al., Perspectives in Developmental Neurobiology 5:247-259, 1998) exposure to hephtachlor may affect neuronal development. To determine the developmental effects of hephtachlor, one group of rats was orally dosed with 0 or 4.2 mg/kg/day of hephtachlor from gestational day (GD) 10 to postnatal day (PND21), and another group was dosed with 0, 0.3, or 3 mg/kg/day from GD10 to PND42. From PND2-5 there was a delay in righting reflex ontogeny in pups exposed gestationally to 3 mg/kg/day of hephtachlor. In adult rats dosed with 3 mg/kg/day of hephtachlor from GD10 to PND42, there was a significant decrease in muscarinic acetylcholine receptor binding in the cerebellum, but not in the striatum or frontal cortex. In adult rats dosed with 4.2 mg/kg/day of hephtachlor from GD10 to PND21, there was a decreased hyperphysiologic response to oxotremorine. An increased response to scopamine on motor activity was observed in adults exposed to 3 mg/kg/day from GD10 to PND42. These data suggest a functional consequence of fewer muscarinic acetylcholine receptors. Thus, exposure to hephtachlor during development resulted in a developmental delay and long-lasting alterations of the cholinergic system. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

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EFFECTS OF CHRONIC BLOCKADE OF SODIUM CHANNELS AND NMDA RECEPTORS DURING DEVELOPMENT ON THE ACQUISITION AND PERFORMANCE OF SEVERAL COGNITIVE FUNCTION TASKS IN RATS.


The effects of MK-801 (a relatively specific NMDA receptor antagonist), phentolamine (a muscarinic receptor blocker) and a combination of the two on the acquisition and performance of several complex, food-reinforced operant behaviors were assessed in juvenile female rats. Audio/visual discrimination (AVD), learning (LRN), and motivation (MOT) were modeled using conditioned position responding, incremental repeated acquisition and progressive ratio tasks, respectively. LO and HI doses of the drugs 0.1 and 1.0 for MK-801, 30 and 150 mg/kg/day for phentolamine, PHT) and LO and HI dose combinations (COMB) of both drugs were administered once daily, 7 days/week by oral gavage beginning on postnatal day (PND) 23. M-Behavioral assessments began on PND 27 and are ongoing (>PND 122). Over the duration of the study, HI MK-801 and HI COMB subjects exhibited decreased body weight gain and performance in all three behavioral tasks (the graphic analysis of body weight gain of all other groups was similar to that of controls. For these two affected groups, response rates were decreased in all tasks, indicating decreased motivation and/or motoric capabilities. Accuracy was decreased for both these groups in the AVD task, but only for the HI MK-801 group in the
DEVELOPMENTAL EXPOSURE TO A BROMONITRATED FLAME-RETARDANT: 2, 2', 4', 4', 5', 5'-HEXABROMO-DIPHENYL ETHER (PBDE 153) AFFECTS BEHAVIOUR AND CHOLINERGIC NICOTINIC RECEPTORS IN BRAIN OF ADULT MICE.


Flame-retardants are used to suppress or inhibit combustion processes in order to reduce the risk of fire. One class of flame-retardants, polybrominated diphenyl ethers (PBDEs), have been found to increase in the environment and in human mothers' milk. In recent studies we have seen that neonatal exposure to some brominated flame-retardants, such as PBDE 47, PBDE 99 and PBDE 209, can cause permanent alterations in spontaneous behavior, an effect that worsens with age. PBDE 99 and PBDE 209 have also been shown to induce these effects during a defined critical phase of neonatal brain development. In view of an increasing amount of PBDEs in mother's milk and in the environment, the present study was undertaken to investigate whether PBDE 153 can induce persistent neurotoxic effects on spontaneous behaviour, learning and memory and cholinergic nicotinic receptors in mice. Mice were exposed to 0.45, 0.9 or 9.0 mg PBDE 153/kg body weight on postnatal day 10. Spontaneous behaviour was observed in 2-, 4- and 6-month-old mice, and mice exposed to 0.9 or 9.0 mg PBDE 153/kg body weight on postnatal day 10 showed significantly impaired spontaneous motor behaviour at all three testing occasions. This effect worsened with age. At 6 month of age learning and memory was assessed in a Swim maze of Morris water maze type. The ability to locate the platform was significantly impaired in animals neonatally exposed to 0.9 or 9.0 mg PBDE 153/kg body weight, compared to control animals. The mice were sacrificed at 6 months of age and analyzed for cholinergic nicotinic receptors in hippocampus using Bungarotoxin. There was a significant decrease in the density of specific [H]-α-Bungarotoxin binding sites in the hippocampus in mice exposed neonatally to 9.0 mg PBDE 153/kg body weight. In conclusion, neonatal exposure to PBDE 153 induces behavioural derangements in adult mice as well as defects learning and memory. In addition, the cholinergic nicotinic receptors in hippocampus were affected.

INTERACTIVE EFFECTS OF METHYLMERCURY AND PCB MIXTURES ON NEURODEVELOPMENT IN MICE.

S. Lee and B. S. Yang. Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Environmental Health, Colorado State University, Fort Collins, CO.

To examine the effects of methylmercury (MeHg) and PCB congeners either alone or in combination on neurodevelopment, several neuronal proteins were measured in the neonatal mice at different time points. From gestation day 10 until postnatal
PERINATAL EXPOSURE TO A POLYBROMINATED DIPHENYL ETHER MElXURE (DE-7I) DISRUPTS THYROID HORMONES BUT NOT NEUROBEHAVIOURAL DEVELOPMENT.

M. M. Taylor', J. M. Hedge', J. L. Deve',1 and K. M. Crofton.1 Neurotoxicology Division MD-74B, NIEHS, ORD, USEPA, Research Triangle Park, NC and Experimental Toxicology Division, NIEHS, ORD, USEPA, Research Triangle Park, NC.

Polychlorinated dibenyl ethers (PBDEs), produced commercially as mixtures, are used as flame-retardants for numerous consumer products. Because of their lipophilic persistence and persistence, PBDEs have become ubiquitous environmental contaminants. Previous work in our Lab has demonstrated that short-term (4 day) in vivo exposure to DE-71 in young rats (a commercial polybrominated diphenyl ether mixture containing mostly tetrabromo- and pentabromo diphenyl ethers) induces hypothyroidism. In this study, motor and auditory function were measured in offspring following perinatal maternal exposure to DE-71. Primiparous rats were orally administered DE-71 (0, 1.1, and 10 mg/kg/day) in corn oil from gestation day 6 (G6D) to postnatal day 21 (PN21). Offspring were evaluated at various ages for body weight, thyroid hormones, hepatic enzymes, eye opening, survival, motor activity development, and auditory startle response. There was no effect of DE-71 on body weight gain or survival in either the dams or offspring. There were dose-dependent decreases in thyroxine (maximal decrease of 55% on PND14), and increases in hepatic glucoruronidation (3G-63). Both endpoints returned to control levels by PN26. There were normal age-dependent changes in motor and sensory behaviors, but no effects of treatment. Benchmark dose (BMD) modeling resulted in BMDs of 2.3 and 5.5 mg/kg/day, and BMDLs (lower bound) of 0.9 and 3.4 mg/kg/day, for serum T4 and hepatic glucuronidation, respectively. These data suggest that DE-71 alters thyroid homeostasis during development, but does not seem to alter sensory or motor development as determined by these endpoints. This abstract does not necessarily reflect USEPA policy.

LONG LASTING NEUROSTRUCTURAL CONSEQUENCES IN THE RAT HIPPOCAMPUIS BY DEVELOPMENTAL EXPOSURE TO A MIXTURE OF POLYCHLORINATED DIPHENYLS.


1 Neuro-Cognitiv Res Lab, Columbus, OH, 2 NIEHS, NIH, Research Triangle Park, NC and 3 Neurotox Div, USEPA, Research Triangle Park, NC.

The objective of the study was to assess the effects of developmental exposure to a commercial mixture of PCBs (Aroclor 1254) on neuronal dendrite morphology of hippocampal CA1 pyramidal neurons in postnatal day (PND) 22 and PND 60 male Long-Evans rats. Rat pups were exposed (once) in utero to Aroclor 1254 (AAPS Standard Inc., Lot # 124-191; 0 and 8 mg/kg/day) from gestational day 6 through PND 21. Thus, pups were exposed to PCBs in utero and through weaning. Male rats (N = 5–6 per group) were sacrificed on PND 22 and PND 60. Brains were formalin-fixed for Rapid Golgi staining of tissue blocks; coded slices of hippocampus were prepared. For branching and spine analysis, 6 CA1 pyramids were randomly selected from each brain. CA1 pyramids were then imaged using the Sholl method of concentric circles. For spine analysis, counts were made along internal and terminal tip segments of 6–7 neurons from each brain. Results of the branching analysis for the PND 22 PCP-exposed rats showed that, compared to controls, there was significantly less dendritic branching in the outer 3/4ths of the dendritic material in the distal 2/3rds of the tree (p < 0.002, Wilcoxon test). Spine analysis also showed a reduction in spines on the terminal tips segments of 22 day old PCP-exposed rats (p < 0.005, T-test). These results suggest that perinatal exposure to Aroclor 1254 resulted in morphometric changes in hippocampus. By PND 60, spine density on terminal tip segments had returned to normal levels. However, branching analysis now showed that compared to controls there was an extensive amount of dendritic material in the distal 2/3rds of the tree (p < 0.001, Wilcoxon test). This suggested a possible structural "hyperplasticity" in neurons damaged by PCP exposure during the developmental period with a residual long-term dysmorphic impact on hippocampal circuitry. (This abstract does not necessarily reflect USEPA policy.)

DEVELOPMENTAL NEUROTOXICITY (DNT) STUDIES WITH TRIFUBOS (DEF) AND METHAMIDOPHOS.

L. P. Sheets, H. E. Hoss and S. G. Lake. Toxicology, Bayer Corp., Wilsall, KS.

DNT studies (OPPTS 870.6300) were conducted with two organophosphorus (OP) compounds in response to a data call-in notice issued by the USEPA. Each OP was administered via the diet at 4, 40, and 400 ppm for DEF and 1, 10, and 30 ppm for methamidophos to male Wistar rats (n=20/level) from gestation day (GD) 0 through postnatal day (PND) 21 for the offspring. The offspring were evaluated for clinical signs, weight gain, sexual maturation, motor activity (figure-8 maze), auditory startle habituation, associative learning and memory (M-maze and passive avoidance), neuropathology and brain measurements. Whole-brain, ecrine, and plasma cholinesterase (CChE) activities were measured in the dams on LD 21 and in the pups on PND 4 and 11 again on PND 21. Slight CChE inhibition in the dam was the only effect at 40 ppm with DEF and 1 ppm with methamidophos. In pups, CChE inhibition occurred at the mid- and high-dietary levels of methamidophos and at the high dose only with DEF. DEF's in pups at the highest doses included decreased weight gain and decreased motor activity during treatment, a reduced startle response on PND 22 and delays in vaginal patency (both OPs) and preputial separation (DEF only). With the high dose of DEF, absolute (but not relative) brain weight was reduced on PND 11 but not on PND 21 or 70. The only effect that persisted to term on PND 70-79 was a lower body weight at the high dose with DEF. Measures of cognitively and habituation (motor activity and startle) were not affected and neuropathology was not evident with either OP at any dietary level. The overall NOELs in the dam and offspring were 4 ppm and 40 ppm, respectively, for DEF and <1 ppm and 1 ppm, respectively, for methamidophos. The overall NOEL in each study was based on inhibition of CChE activity in the dam. This outcome and the resulting overall NOELs are consistent with those of the two-generation reproduction studies.

CONTROL BEHAVIORAL DATA FROM DEVELOPMENTAL NEUROTOXICITY STUDIES (DNT) AND PERI-PERNATAL STUDIES: IS SEXUAL MATURATION IN DNT STUDIES IMPROVED BY ANALYZING THREE PUPSEXUNIT.


Behavioral evaluations of the pup and adult rat (motor activity, acoustic startle habituation, sexual maturity) are requirements of DNT studies conducted under USEPA guidelines (OPPTS 870.6300). Learning and memory (i.e. passive avoidance and watermaze) and sexual maturity have been conducted for perinatal studies since the mid-80s. The ranges of normal values for the various parameters have not been published. The behavioral values for control rats participating in nominally identical DNT and approximately 60 pharmaceutical testing studies have been compared. The results of approximately 40 control studies were presented in 2000. Premature Sprague-Dawley rats were administered the selected substances orally beginning on Day 0 of gestation and continuing through Day 11 or 21/2. Control data are from the same day of gestation and continuing through Day 11 or 21/2. Control data are from the same day of gestation and continuing through Day 11 or 21/2. Control procedures were based on the demonstration of sexual maturity (female rats beginning on DP 28 and males beginning on DP 39). The availability of such control data for behavioral measurements will help clarify the extent to which between subject and between litter (sexual maturation) variation affects the statistical power of comparisons between treatment and control groups in DNT studies.
NEUROMORPHOLOGICAL EVALUATION OF CONTROL DATS FROM SEVEN DEVELOPMENTAL NEUROTOXICITY STUDIES (DNT) INCLUDING POSITIVE CONTROL DATA.


Quantitative morphometric measurements of the pup and adult rat brain are a requirement of DNT studies conducted under USEPA guidelines (OPPTS 870.6300). Relatively few guideline studies of this type have been conducted to date, and the range of normal values for the various parameters has not been previously established. The morphometric values for control rats participating in seven nominally identical DNT studies were compared. Pregnant Sprague-Dawley rats were administered control substances orally beginning on Day 0 or 5 of gestation and continuing through Day 11 or 21/22 of lactation (Day of birth = DP 1). Brains from DP 12 pups and adult rats were weighed, and the gross anterior-posterior lengths of the cerebrum and cerebellum were recorded. Microscopic measurements of the frontal cortex, parietal cortex, hippocampus, gyrus, corpus callosum and cerebellum were performed and recorded. Qualitative neuropathological evaluations were performed on the DP 12 rat brain and the adult rat brain, spinal cord, dorsal root ganglia, Cessian ganglia and associated trigeminal nerve, and selected peripheral nerves. Morphometric measurement, collected during a validation study, comparing Day 10 and 12 Sprague-Dawley rat pups are provided. The results demonstrate that the measurement data collected from the selected neuroanatomical areas reveal age-related developmental differences that might, therefore, be predictive of either an in utero neurotropic effect or a delay in brain development. The availability of control as well as positive control data for morphometric measurements will help clarify the extent to which between-subject variation affects the statistical power of comparisons between treatment and control groups in DNT studies.

THE RELIABILITY AND SENSITIVITY OF HISTOMORPHOMETRIC METHODS IN DETECTING MORPHOLOGICAL CHANGES IN THE DEVELOPING RAT BRAIN.


Pathology, Clinic/Path BioResearch Ltd., Seneville, PQ, Canada and Toxicology, Avena CropScience, Research Triangle Park, NC.

In regulatory developmental neurotoxicity studies the study guidelines require that morphometric analyses be conducted on the developing rat brain. Histomorphometric evaluation is recognized as a useful tool to quantify gross changes and histomorphological changes in the developing brain; however, it is important to identify and examine regions of the brain that are sensitive to toxicological insult. Further, high quality sections and consistent histomorphometric techniques are essential for reliable quantitative data. The developing rat brain undergoes rapid growth and development during the first 2 weeks after birth. However, certain morphological changes would be anticipated during this time. The objective of the present study was to ensure that the histomorphometric procedures employed in our laboratory are sensitive enough to detect the subtle morphological changes occurring in the developing brain. For this we compared the morphometry of various brain regions from 10 day and 13 day old rats. The brains (6-8/age) were immersion fixed in 10% neutral buffered formalin, embedded in paraffin, and stained. Histomorphometric evaluations were made by the same operator using a BIOQUANT/TCW image analysis system (BIOQUANT R & M Biometrics, Inc.) on one section of forebrain (left and right frontal cortex; left and right parietal cortex; corpus callosum) and cerebellum (lobule base thickness; internal and external granular layer thickness) from each animal. Significant differences between 10 and 13 day old rats were detected in the frontal and parietal cortex (males only), the thickness of the base of cerebellar lobule 6/7/8 and lobule 9 (females only), and in the internal and external granular cell layers of the cerebellum (males and females). Our results indicate that during the second week of postnatal development the internal and external granular layers of the cerebellum are the most sensitive and consistent brain regions that can be measured reliably.

DEVELOPMENTAL NEUROTOXICITY TESTING GUIDELINES: A QUALITATIVE RETROSPECTIVE ANALYSIS OF POSITIVE CONTROL DATA.


Health Effects Division, OPPTS, USEPA, Washington, DC and Neurotoxicology Division, NHEERL, ORD, USEPA, Research Triangle Park, NC.

The USEPA Developmental Neurotoxicity (DNT) Study Test Guideline calls for both functional and neuropathological assessments in offspring during and following maternal exposure. This guideline also requires data from positive control (PC) agents. Submission of these data permit evaluation of laboratory proficiency in the detection of changes in the structure and function of the developing nervous system and comparison of the sensitivity of assessments in different studies and labs. This project surveyed approaches taken in contract and industrial laboratories in generating and providing these data. Data from DNT studies conducted by 13 different laboratories were summarized by method, e.g., motor activity, for information on: age relevance, Dose-Response, gender, group size, statistics, report quality, age assurance, age of data relative to main study, and relevance of methods to the main study. Endpoints included: developmental landmarks, FOB, motor activity, startle response, learning and memory, standard histopathology and brain morphology. Results ranged from no PC data for two laboratories, to our laboratory which submitted 16 different reports. The qualitative range was similarly broad, from excellent to extremely poor. Various quality problems were identified including poor report structure (e.g., copies of poster presentations), lack of individual data, inadequate methodological details, differences in methods between the PC report and the main study, submission of very old data or data from completely different laboratories, significant variance in the submitted data, use of inappropriate PC chemicals or doses that were without effect, lack of statistical analysis, use of only one sex, and use of inappropriate age animals. Preliminary analyses suggest that there were only 4 out of 13 laboratories judged to have submitted adequate PC data. This abstract does not necessarily reflect the policy of the USEPA.

PRENATAL NICOTINE EXPOSURE EVOKES LONG-TERM ALTERATIONS OF CELL STRUCTURE IN HIPPOCAMPUS AND SOMATOSENSORY CORTEX.

T. S. Roy, F. J. Seidler and T. A. Sladkin.

Pharmacology & Cancer Biology, Duke University Med. Ctr, Durham, NC.

Offspring of women who smoke during pregnancy show behavioral abnormalities including increased incidence of attentional deficit, learning disabilities and cognitive dysfunction. Animals models indicate that nicotine elicits changes in neural cell replication and differentiation, leading to deficits in synaptic neurochemistry and behavioral performance, many of which first emerge at adolescence. In this study, we evaluated cellular morphology and regional architecture in the juvenile and adolescent hippocampus and the somatosensory cortex in rats exposed to nicotine prenatally. Prenatal rats were given nicotine throughout gestation via minipump infusion of 2 mg/kg/day, a regimen that elicits nicotine plasma levels comparable to those found in smokers. On postnatal days 21 and 30, brains were perfusion-fixed, coronal slices were taken between the anterior commissure and median eminence, and the morphology of the dorsal hippocampus and somatosensory cortex were characterized. In the hippocampal CA3 region and dentate gyrus, we found a substantial decrease in cell size, with corresponding decrements in cell layer thickness, and increments in cell packing density. Smaller, transient changes were also found in CA1. In layer 5 of the somatosensory cortex, although there was no significant decrement in the average cell size, there was a reduction in the proportion of medium-sized pyramidal neurons and an increase in the proportion of smaller, somatopyramidal cells. Taken together, these data suggest that prenatal nicotine exposure compromises neuronal maturation, leading to long-lasting alterations in the structure of key brain regions involved in cognition, learning and memory. (Supported by R01 DA14247 and a fellowship from IBRO)

NICOTINE AFFECTS ZEBRAFISH MOTONEURON DEVELOPMENT.


Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO and Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, CO.

Nicotine is a drug of abuse that has a profound impact on society. Of great concern is the harm that is caused when a pregnant mother smokes and exposes her unborn child to nicotine. Nicotine has been shown to be a developmental toxicant in several experimental models, but the mechanism by which nicotine alters development is not well understood. It is thought that the actions of nicotine are mediated by the nicotinic acetylcholine receptors (nAChRs). We are utilizing zebrafish embryos to gain insight into how nicotine modulates development with a particular emphasis on nervous system development. For these studies, zebrafish embryos were exposed to 33 micromolar nicotine beginning at 22 hours post-embryon (hpe). At this dose, embryos do not respond to touch (at 48hpf) and are functionally paralyzed. At 48 hpf, nicotine had no apparent effect on the gross morphology of the embryo. However at 66 hpf, and 120 hpf, embryos exposed to nicotine were 69% and 76% shorter in length, respectively, compared to controls. The morphological deficit coupled with the behavioral deficit suggests that motor or neuromusculature development was altered by nicotine. In order to investigate these possibilities, we imaged living transgenic zebrafish that express green fluorescent protein (GFP) in their spinal secondary motoneurons. Embryos exposed to...
nicotine had significantly fewer GEP positive neurons than stage matched controls. This effect was partially reversed by 2 mM MLA, a nicotine antagonist known to act at the α7 receptor subtype. Furthermore, 20 mM DHE, an α4/α2 subtype specific nicotine receptor antagonist also reversed the nicotine effects. These results demonstrate that nicotine is an excellent vertebrate model to study developmental toxicity, and suggest that an "α2/4 receptor 2-like" αCβH subtype may be involved in mediating nicotine's actions in zebrafish early life stages.

655 PHARMACOKINETICS OF SB-247083, A POTENT AND SELECTIVE ENDOTHELIN-A RECEPTOR ANTAGONIST, IN THE RAT, DOG, AND MONKEY.


The endothelins (ET) are among the most potent vasoconstrictors identified to date, and have been implicated in such diseases as renal failure, pulmonary hypertension, atherosclerosis, and congestive heart failure. There is currently intense interest in developing selective antagonists of the ET-A subtype receptor, and one such antagonist is SB-247083 ((E)-1-buoyl-5-(2-carboxyphenyl) methoxy-4-chlorophenyl)-1H-pyrazole-4-carboxylic acid). 2-Propanolic acid. This compound has been shown to evaluate the preclinical pharmacokinetics of SB-247083. Clearance of SB-247083 was low in the rat, moderate in the monkey, and high in the dog. Clearance is linear in the rat, with modest evidence of nonlinear elimination at high concentrations in the dog and monkey. First-pass hepatic extraction was approximately 60% in the rat, and ranged from 30 to 50% in the monkey. Oral bioavailability of SB-247083 administered as a solid formulation was good in the rat (24%), low in the dog (1 to 8%) and the monkey (2%). Pre-administration of 0.1 N HCL to normalize the zebrafish fasted dog stomach did not improve bioavailability. Various formulations and salt forms of SB-247083 were evaluated; the most favorable formulation was the acetic acid salt, which appeared to be a potent orally bioavailable in each species tested. These data suggest that SB-247083 may be a promising ET-A receptor antagonist, and may have utility in various ET-mediated diseases.

656 KINETICS AND LOCOMOTOR ACTIVITY FOLLOWING COCAINE AND KETAMINE ADMINISTRATION IN RAT.

H. Ruedif and M. Abdel-Bakih. Pharmacology & Physiology, GSB, UMDNJ, Newark, NJ.

The co-abuse of cocaine (COC) and ketamine (KET) occurs with high frequency. The presence of another active substance with COC allows the potential of various drug-drug interactions to occur. COC metabolism may play an important role in its toxicity. Buprenorphine (BEP) has been shown to be a potent vasoconstrictor and convulsant agent. This study investigated the plasma time course, tissue distribution and locomotor activity of COC or KET alone and their combination in rat. COC (5 mg/kg i.p.), KET (100 mg/kg p.o.) or KET followed by COC (same doses and routes of administration) were utilized. KET administered concurrently with COC significantly reduced COC bioavailability in the plasma. The AUC and elimination half-life, while the oral clearance was increased versus COC alone administration. The AUC of plasma BE was significantly increased in the combination treatment compared to COC alone. Tissue distribution study revealed that, the brain, heart and liver contents of COC and norcocaine were significantly lowered at 5, 15 and 30 min following KET administration versus COC alone. However, in the same organs, BE was significantly higher in the combination group compared to COC alone. On the other hand, COC treatment did not affect the kinetics of KET as a parent compound. Locomotor activity study showed that, rats treated with COC and KET in combination had a significantly lower horizontal locomotor activity compared to COC treatment. The results suggest that KET increases the metabolism compared to COC treatment. The high plasma and tissue concentrations of BE after the combination treatment, which is potent cardiovascular and neurotoxic agent, may possibly affect the toxicological profile of COC as well. (Supported in part by the Egyptian government).

657 PLASMA PHARMACOKINETICS OF TRICLOSAN FOLLOWING REPEATED DERMAL EXPOSURE.


Triclosan (TCS, 2, 4, 4'-trichloro-2'-hydroxydiphenyl ether) is an anti-microbial agent that has been used for over 30 years in a variety of personal care products. This study evaluated the steady-state plasma concentration and pharmacokinetic profile of TCS following repeated dermal application. Plasma levels of TCS were followed in 13 human volunteers (7 males and 6 females) after use of a commercial hand wash containing 1% TCS, 6 times per day for 20 days. Volunteers were instructed not to use any other TCS-containing products during the course of the study. Blood samples were taken prior to first hand washing on study day 1 (baseline TCS level) and study days 5, 10, 15, and 20. Blood samples were also taken post exposure on study days 1, 2, 3, and 30. Samples were then analyzed by LC/MS/MS for free and total (conjugated) TCS in plasma. On day 20, the subjects were requested for 12 hours, during which hand washing continued every 2 hours and blood samples obtained prior to each hand wash. Pharmacokinetic analysis of the plasma concentration data supports a first order, single compartment model for TCS. Steady state levels of free and total TCS were reached by study day 5. Mean ± SD steady state levels of free and total TCS from day 5 to day 20 ranged from 2.0 ± 0.005 to 10.6 ± 1.6 ng/ml and 67.1 ± 34.7 to 79.4 ± 48.9 ng/ml respectively. Maximum free and total TCS plasma levels during the study for any volunteer were 69.9 and 229 ng/ml. The average elimination half life and elimination rate constant, calculated from the individual values for the steady state population, were 1.4 days and 0.615 day^-1. Overall, the study results show that TCS is absorbed through the skin and rapidly eliminated from the body. Furthermore, the data support that human exposure to TCS resulting from continued use of a hand wash containing 1% TCS is significantly below exposures associated with animal toxicity end points.

658 APPLICATION OF THE CULEX™ AUTOMATED BLOOD SAMPLER IN THE PHARMACOKINETIC STUDY OF SU006668 IN AWAKE RATS.


Conventionally in the in vivo rat study of pharmacokinetics, a series of blood samples are collected manually through a dwelling intracardiac catheter. A novel robotic device, Culex™, automated blood sampler (Dyna Analytical Systems, Inc., West Lafayette, IN), provides an alternative way to automatically collect blood from awake and freely moving rats. The purpose of this study was to evaluate the feasibility of the Culex™ automated blood sampler in the application of pharmacokinetic studies in rats. SU006668, an anisotacticenetic drug for Phase I clinical development for the treatment of solid tumors, was studied as a model drug for this purpose. Fifteen rats received intravenous (i.v.) or oral (PO) administration of the drug. For each group, the blood samples were collected up to 12 hours after manual blood collection method (n=4 for both IV and PO groups) or by using Culex™ automated blood sampler (n=5 for IV and n=4 for PO). The plasma concentrations of SU006668 were determined using a LC/MS/MS method. The pharmacokinetic parameters were determined by non-compartmental analysis using WinNonlin Professional Edition. The results shown below indicate that the pharmacokinetic parameters obtained from two blood sampling techniques (Culex vs Manual) were comparable (P>0.05, t-test), and consistent with the historical pharmacokinetic data on SU006668. The study suggests the feasibility of blood sampling by the Culex system for the study of pharmacokinetics.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>IV, Manual</th>
<th>IV, Culex</th>
<th>PO, Manual</th>
<th>PO, Culex</th>
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<tr>
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<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<td>25905 ± 3872</td>
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<td>1.6 ± 0.3</td>
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<tr>
<td>Tmax (hr)</td>
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<td>2.3 ± 0.3</td>
<td>2.4 ± 0.8</td>
<td>2.1 ± 0.3</td>
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<tr>
<td>Cmax (ng/mL)</td>
<td>12738 ± 2052</td>
<td>1245 ± 6149</td>
<td>9.5 ± 19</td>
<td>5.5 ± 19</td>
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</table>

659 CORTICOSTERONE IN DRINKING WATER ENHANCED THE ABSORPTION OF A SINGLE ORAL DOSE OF CORTICOSTERONE.

T. Tong, K. Fahrman and M. Ehrlich. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Corticosteroids are stress indicators that contribute to many body functions. Effects of corticosterone exposure on corticosterone kinetics were studied in adult male Long Evans rats. Some of these were gavaged with corticosterone in corn oil at 20
CONTINUOUS INFUSION IN MARMOSET MONKEYS: FEASIBILITY OF A PORT-CATHETER TECHNIQUE.


The common Marmoset (Callithrix jaccus jaccus) can play an important role as the second species in the safety evaluation of new drugs. Hence the need occurred to evaluate the suitability of this primate as a model for continuous infusion studies. This work investigates the feasibility of a port-catheter technique in marmosets and concentrates on the technical details of the surgical implantation procedure. The right and the left thigh as well as the abdominal wall and the dorsal region of the thorax are shaved. The operation area in the upper field of the right thigh is sterilized with Betadine solution. Following intramuscular premedication with Ketamine, Xylazine and Atropine, anesthesia of the marmoset is performed by inhalation narcosis with Isoflurane. During the operation process, breathing is supported using a respirator. The proximal section of the V. femoralis is isolated for access to the venous system. The silicone catheter (3 French O.D.) is placed close to the V. femoralis sin. and the disconnected distal V. femoralis is used as a point of fixation. A subcutaneous pocket in the region of the upper thigh is used to fix the externally fixed titanium port (Type POPS II), placed on the animal's back, with the injection site. The outer port is fixed on the skin by a patch, the silicone membrane of the port is directed cranially to inject substances easily. To protect the surgical operation area and to support wound healing, a tube-shaped wound cover is used to exclude thorax and abdominal body. The suture material is removed after 8 days. A heparin solution of 5 IU/mL is regularly used to rinse and protect the infusion system. In conclusion, a port-catheter system is feasible for marmoset monkeys since it is shown here that such a system can successfully be implanted in these very small non-human primates. First experiences confirm successful and intermittent use of the system for more than six weeks.

INCREASED PLASMA LEVEL OF ACETAMINOPHEN-GLUCURONIDE IS ASSOCIATED WITH INDUCTION OF MULTIDRUG RESISTANCE PROTEIN 3 (MRP3) IN LIVER.

A. A. Sitt, N. J. Cherington, J. M. Maher, and C. D. Klaassen. Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS.

Previous studies report that treatment with the cyclochrome P450 2B1/2 inducers phenobialdehyde (PB) and thioacetamide (TSA) can decrease biliary excretion of acetaminophen-glucuronide (AA-GLUC) and increase excretion of AA-GLUC into blood. The canalicular Multidrug Resistance Protein 2 (Mrp2) can transport glucuronide conjugates into bile, whereas the sinusoidal protein Mrp3 is thought to transport these conjugates into blood. Hence, it is hypothesized that Mrp2 and Mrp3 are responsible for the excretion of AA-GLUC conjugate into bile and blood. Moreover, PB and TSA-induced hapatocellular necrosis and biliary excretion of AA-GLUC may occur via increased hepatic Mrp3 levels. Therefore, the goal of this study was to determine 1) whether PB and TSA, compounds known to increase plasma concentrations of AA-GLUC up-regulate Mrp3 expression, and 2) whether compounds that induce Mrp3 expression, such as dithiole sulfox (DAS) and olsipront (OLT), can correspondingly decrease biliary and increase hepatocellular excretion of AA-GLUC. Sprague-Dawley rats were administered PB, TSA, OLT, or TSA, on a vehicle for four days. To determine the effects of these chemicals on AA excretion, the carotid artery and bile duct were cannulated and blood and bile were collected at various times up to 6 hr after AA injection into the carotid cannula. AA-GLUC content in bile and blood was quantified by HPLC and Mrp2 and Mrp3 mRNA was quantified using the branched DNA signal amplification assay. Interestingly, all of the chemicals increased the hepatocellular and decreased the biliary excretion of AA-GLUC. Furthermore, all of the compounds dramatically increased hepatic Mrp3 mRNA by at least 5 fold, but had little or no effect on hepatic Mrp2 mRNA levels. Together, these studies correlate increased Mrp3 mRNA levels with increased hepatocellular excretion of AA-GLUC and suggest that induction of Mrp3 affects the route of drug excretion. (Supported by NIH grants ES-09716 and ES-07079)

KINETICS OF PROPYLENE GLYCOL MONOMETHYL ETHER (PGME) AND PGME ACETATE (PGMEA) FOLLOWING INTRAVENOUS (IV) ADMINISTRATION TO MALE FISCHER 344 RATS AND THE IN VITRO HYDROLYSIS OF PGMEA IN RAT AND HUMAN BLOOD AND LIVER HOMOGENATES.


The blood kinetics of PGMEA and PGMEA following iv administration to rats was conducted to determine toxicological equivalency between the two materials. The in vitro rates of hydrolysis of PGMEA to PGME in incubations of rat and human blood as well as liver homogenates were measured for use in a future PB-PK model for PGMEA. For investigation of the kinetics of PGMEA or PGMEA following iv administration to rats, equivalent doses of 10 mg PGMEA/kg or 14.7 mg PGMEA/kg and 100 mg PGMEA or 127 mg PGMEA/kg were used. Blood samples were drawn from the jugular vein canula through 12 h post-dosing and analyzed for PGMEA and PGMEA. Plasma time course of PGMEA following iv administration of 10 mg PGMEA/kg or 14.7 mg PGMEA/kg were identical similarly for the high dose of 100 mg PGMEA/kg or 147 mg PGMEA/kg. Plasma time courses of PGMEA following iv administration of 14.7 mg PGMEA/kg were determined and half-lives were 1.55 and 3.37 min, respectively. Incubations were conducted through 6 h at concentrations of 5 and 50 mg PGMEA/mL. Samples were analyzed via GC or LC-MS/MS for determination of PGMEA and PGMEA in blood or liver homogenate incubations. The disappearance of PGMEA was faster in rat than in human blood samples. The hydrolysis half-lives were 36 and 34 min for the 5 and 50 pg/mL concentrations of PGMEA in human and rat blood, respectively. The half-lives were 16 and 15 min, respectively. The rate of loss of PGMEA was similar in rat and human liver homogenates incubations. Hydrolysis half-lives were 27 and 30 min for the 5 and 50 pg/mL concentrations of PGMEA in human liver homogenate, whereas in rat liver the hydrolysis half-lives were 34 min for both concentrations. These data indicate that PGMEA is rapidly hydrolyzed in the body to its parent glycol ether, PGME. Once hydrolyzed the kinetics for PGME derived from PGMEA is identical to that for PGMEA. (Supported by the American Chemistry Council Glycol Ether Panel.)

DISPOSITION AND ORAL BIOAVAILABILITY OF HALOACETATE MIXTURES IN F344 RATS.

S. A. Saghri and L. R. Shultz. Battelle PNL, Richland, WA.

Chloro, bromo and mixed bromo- chloro haloacetates (HAs) are by-products of drinking water disinfection and exhibit both carcinogenic and reproductive/developmental toxic properties. Drinking water typically contains a mixture of di- and tri-HAs. Recent toxicokinetic studies have identified significant differences in the disposition of di- and tri-HAs. It is now established that di-HAs are almost exclusively eliminated via metabolism that occurs as a glutathione dependent process. The hydrolysis half-lives of di-HAs are greatly reduced after prolonged exposure due to suicide inactivation of the GST-protein. Preliminary evidence suggests that the tri-HAs do not exert this effect. We measured the tissue distribution, in vivo plasma/tissue partitioning and oral bioavailability of two separate HA mixtures containing both di- and tri-HAs in F344 rats before and after depleting GST-zeta activity. Results indicated the plasma/tissue partition coefficients ranged between 0.6-1. The oral bioavailability of tri-HAs was not affected by GST-zeta depletion and ranged between 67-92%. The oral bioavailability could not be assessed for the di-HAs as the area-under-the-curve was always greater after oral dosing compared to the iv reference dose. This phenomenon appears to be due to secondary peaks that appear in the plasma profiles of di-HAs after oral dosing. Supported by EPA STAR grant R825954.

STEREOSPECIFIC DISPOSITION OF BROMOCHLORO- AND CHLOROFLUOROACETATE: EFFECT OF GST-ZETA DEPLETION.

L. R. Schults and S. R. Sylvester. Battelle PNL, Sequim, WA.

The chloro- and bromo- haloacetates are drinking water disinfection by-products and rodent carcinogens. Chloro, bromo di-haloacetates are also mechanism based inhibitors of glutathione-S-transferase zeta (GSTZ2-1). We studied the stereoselectivity.
cific toxicokinetics of two chiral di-haloacetates in male F344 rats (-)-, (+)-bromochloroacetate (BCA), and racemic chlorofluoroacetate (CFA), a non GST-zeta inhibiting di-haloacetate. These experiments were repeated in animals that had previously been treated with dichloroacetate (DCA) to deplete GST-zeta activity. Results indicated that in native rats the elimination half-life of (-)-BCA was 0.07 hr compared to 0.40 hr for (+)-BCA. A comparable difference in elimination half-life was also observed for the CFA stereoisomers. 70% of (+)-BCA was absent with both stereoisomers having an elimination half-life of approximately 0.4 hr. This finding was in contrast to results for CFA, which still maintained the same relative difference in elimination rate between its stereoisomers although overall elimination was diminished in GST-zeta-depleted rats. The AUC(0-CEx) of (-)-BCA after oral administration differed by more than 10 fold in native rats but this difference was reduced considerably in GST-zeta-depleted rats. The oral bioavailability varied between 20-67% for the different BCA stereoisomers. These results indicate (+)-BCA is a poor substrate for GST-zeta and the internal dosimetry of (-)-BCA, (+)-BCA will differ considerably after drinking water exposure. Supported by EPA STAR grant R825954.


The toxicokinetics (TK) of hexachlorobenzene were assessed after p.o. (300 mg/kg) and i.v. dosing (100 or 165 mg/kg) to female Sprague-Dawley rats. TK parameters estimated for the p.o. group included maximum blood levels (Cmax) and time to peak (Tmax), elimination rate (k) and half-life (t1/2), volume of distribution (V/F), clearance (Cl/F), dose-normalized areas under the concentration-time curve (DNAUC), and bioavailability (F). Parameters estimated for the i.v. groups were Cmax distribution and elimination rates (k and b) and t1/2 volumes of distribution (V), clearance (Cl), and DNAUC. Liver, fat, and lung were also analyzed for HCB. Kinetic parameters estimated included mean residence time, Cmax, Tmax, t1/2, AUC, and DNAUC. Blood levels of HCB showed linear kinetics at the i.v. doses used, and HCB given orally was completely and rapidly absorbed (F = 1.2, T = 3 hr). TK parameters estimated for the p.o. and i.v. groups were comparable except for Cl. The fact that the data collection period covered only 3 to 4 elimination half-lives, and the final portions of the blood concentration-time curves were flat may explain this. TK parameters for HCB in fat and liver behaved in a similar manner, and suggest linear kinetics over the dose range. HCB was highly concentrated in fat. TK parameters estimated for HCB levels in lung suggest non-linear kinetics. TK parameters for the oral group are consistent with those from an earlier study, where dose normalized peak blood levels (Cmax) after a p.o. dose of 30, 000 mg/kg were of similar magnitude to those at 300 mg/kg. In the earlier study, Tmax was 3 hr, thus indicating linear (first-order) absorption following an oral dose of 300 to 3000 mg/kg of HCB. Other parameters, such as Cl/F, F, and t1/2 were also similar in magnitude. Bioavailability was found to be complete (F = 1.0) for female rats receiving an oral dose of 30, 000 mg/kg, estimated using the average DNAUC from rats in these low and high i.v. dose groups.


Methofuran is a proximate toxic metabolite of pulegone, a monoterpene constituent of perilla oil, and is believed to account for a significant percentage of pulegone toxicity. Metabolism of methofuran yields a highly reactive y-ketoenal intermediate that covalently binds to protein. A study comparing the disposition of methofuran to pulegone in F344 rats has been conducted as part of the National Toxicology Program initiative in herbal medicines and dietary supplements. Adult male and female F344 rats were given single oral doses of (-)-methofuran (6 or 60 mg/kg) in corn oil; urine and feces were collected and the animals were sacrificed at 24 or 72 hours for tissue collection. Although methofuran is fairly volatile, only 0.22% of the dose was recovered as unchanged compound. Overalls, 92 to 98% of the dose was recovered. Methofuran is excreted in the urine (57 to 69% after 72 hours) and feces (19 to 28%). The highest concentrations of methofuran-derived radioactivity were in blood and liver at 24 and 72 hours. The concentration of radioactivity in kidneys from males was 2-fold higher than those from females at 24 hours. The sex-related difference in kidney concentration is not as great as observed for pulegone, but the results may indicate that methofuran and/or a metabolites binds to 24a global. At 72 hours, 10 to 14% of the dose was still in the tissues. This is 2 times the residual pulegone in tissues at 72 hours at a similar dose.


2-Butoxyethanol (BE) is a widely used solvent in industrial and consumer cleaning and coating products. An extensive toxicity and mechanistic database shows humans are considerably less sensitive than rats and mice to hemolysis, the main endpoint of toxicity. However, a recent NTP inhalation bioassay identified the forestomach and liver of mice, but not rats, as target organs for potential tumorigenicity. A series of studies were conducted to evaluate the role of dosimetry in producing lesions specifically in the forestomach. Results from these studies indicate that forestomach tumors appear to be produced by a chronic irritation/cytotoxicity mechanism. Further questions the relevance of the forestomach tumors for humans which lack this tissue. Irritation and compensatory hyperplasia were induced in the forestomachs of mice upon oral administration of neat BE. Similar, but milder lesions were also produced by systemic administration (intraperitoneal injection) of comparable doses. Salivary excretion was found to be a contributing source of BE and its metabolite, butyroxyacetic acid (BAA) to forestomach tissue in female mice following intraperitoneal injection. Salivary levels of BE in the mice were nearly equivalent to blood concentrations. The elimination half-lives for BE and BAA in blood were 0.06 and 0.9 hr, respectively, which is considerably faster than rats or humans. Based on assumed kinetic and toxicity studies followling inhalation, oral and intraperitoneal injection routes of exposure, sources of BE and its hemolytic metabolite, butyroxyacetic acid (BAA) in the forestomachs of mice can be attributed to a combination of systemic delivery via the blood, elimination in saliva with subsequent oral ingestion and oral absorption to the stomach. BE was deposited on the fur. Taken together, these results are a significant role for local tissue dosimetry in the development of forestomach cytotoxicity in mice. (Sponsored by the Ethylene Glycol Ethers Panel of the American Chemistry Council).


Disposition of two treat C-polymethylsiloxanes (PDMS) with viscosities of 10 and 350 are from the GL tract was evaluated in male and female Fischer 344 rats. A single, oral dose of 1000mg/kg of body weight was administered to rats in Mass Balance (MB: 4 females and 4 males) and Whole Body Autoradiography groups (WBA: 4 females and 4 males). Rats in the MB groups were housed in Ruth-style metabolism cages for collection of excreta and the assessment of the recovery of total radioactivity. At 96 hr post-dose, rats were euthanized by cardiac puncture under anesthesia and blood, liver, GI tract, kidneys, spleen, eyes, peritoneal fat, and remaining carcasses were collected and processed for analysis. Radioactivity content in each sample was measured by liquid scintillation counting (LSC). Aliquots of the fecal homogenates were extracted and analyzed by HPLC, which radiochemical detection using a gel-permeation chromatography column for qualitative determinations of the radioactivity covalent binding profiles. Rats in the WBA groups were sacrificed at 12, 24, 48 and 96 hr post-dose and immediately frozen at -75°C. Sagittal sections of 40 mm in thickness were collected at various levels to include major organs and tissues and exposed to x-ray film -80°C. Both quantitative (LSC) and qualitative assessments (WBA) showed that radioactivity was rapidly extracted through gastrointestinal tract. In assessment of tissue radioactivity distribution by WBA at 12, 24 and 48 hr post-dose showed that radioactivity was concentrated in the contents of the GI tract. No significant radioactivity was detected outside the GI tract. Water contamination of the GI tract with the exception of exterior contamination. Dose recovery from all animals through 96 hr post-dose was > 93.6%. Virtually the entire recovered dose was recovered from excreta (95.0 to 99.0%) with little, if any, absorption. The entire radioactivity recovered in excreta was attributed to the unchanged PDMS, 10 or 350. No detectable degradation products were present in feces. Funded in part by Silicones Environmental, Health and Safety Council.
CHROMIUM ACCUMULATION IN THE TISSUES OF RATS, MICE, AND GUINEA PIGS EXPOSED TO SODIUM DICHLOROMETHYL DIHYDRIDE IN DRINKING WATER FOR TWENTY-ONE DAYS.


1 RTI, Research Triangle Park, NC and 2 NIEHS, Research Triangle Park, NC.

Chromium (Cr) is widely used in industry for corrosion inhibition, metal finishing, manufacturing of safety matches and production of pigments. Hexavalent chromium (VI) has been found to contaminate soil and air. This study was designed to evaluate the relationship between the drinking water concentrations of Cr (VI) as sodium dichromate dihydrate (SDD), and tissue accumulation of Cr in three species. Male Fischer 344 rats, B6C3F1 mice, and Hartley guinea pigs were exposed to SDD in tap water for 2 days at 0, 1, 3, 10, 30, or 100 mg Cr/L water (8, 2.87, 8.02, 28.7, 86.2, and 862 mg SDD/L, n=4/dose). On Study Day 22, all animals received a tap water (0 mg Cr/L) Actual doses received were calculated based on twice weekly water consumption data and weekly body weights. On Study Day 24, animals were humanely sacrificed and blood, kidneys, and femurs were collected. Whole blood and tissues were analyzed for total chromium content. Water consumption remained normal throughout the course of the study for rats and mice and did not differ between dose groups (water consumption could not be calculated for guinea pigs). In mice, mean body weights were not significantly different from controls. However, for both rats and guinea pigs, mean body weights at the 100 and 300 mg/L dose levels were decreased when compared to controls. Cr levels in rat kidney were ca. 3 times higher than in mouse kidney (rat range: 0.6-9.1 µg/g, m; mouse range: 0.1-3.3 µg/g). Cr levels in guinea pig kidney were similar to mouse kidney (range: 0.1-3.1 µg/g). In all three species, Cr tissue concentrations appear to increase in a linear manner up to 80 mg Cr/L. Tissue concentrations of Cr do not appear to increase at higher dose levels. This may be due to a decrease in the bioavailability of Cr or a decrease in the uptake of Cr into specific tissues. Tissues appear to reach a saturation level at the 100 mg Cr/L dose level.

670 BIOAVAILABILITY OF PENTACHLOROPHENOL FROM DIFFERENT TYPES OF SOILS.

X. Z. Pu, Y. Z. Zhao1, L. S. Lee, A. P. Schwab2 and G. P. Carlson3.

1 Health Sciences, Purdue University, West Lafayette, IN and 2 Agronomy, Purdue University, West Lafayette, IN.

Pentachlorophenol (PCP) is a broad-spectrum biocide widely used as a wood preservative and is thus a ubiquitous environmental pollutant. Evidence exists that usually only a fraction of the chemicals in soil is bioavailable to organisms, and this bioavailability is influenced by chemical and soil properties. In the current study, the absolute and relative bioavailability of PCP from soils varying in organic carbon content, particle size and pH were examined. Four uncontaminated soils and two contaminated soils from a wood preserving industry were collected from different sites in Indiana. Uncontaminated soils were spiked with 50 and 100 mg/kg PCP. The PCP concentrations in the soils from contaminated sites was approximately 200 mg/kg. Soils and corn oil containing equivalent levels of PCP were administered to male Sprague-Dawley rats (weight range 275 to 350 g) by gavage at two dose levels—100 and 200 µg/kg body weight. Equivalence doses were also given via intravenous injection (iv). Blood samples were obtained from the abdominal aorta at 1, 3, 6, 12, 24, and 48 hours after administration. PCP concentrations in plasma were analyzed by GC-ECD. Areas under the curve (AUC) were calculated for each soil and compared to those of the iv injection and corn oil groups to estimate the absolute bioavailability (AUC of soil/AUC of iv injection) and relative bioavailability (AUC of soil/AUC of corn oil). Both absolute and relative bioavailabilities were very high for each soil at each dose with no statistical differences detected among them. Bioavailabilities decreased in one of the environmentally contaminated soils. These results indicate that PCP in soil is readily absorbed into the blood from the gastrointestinal tract and the soil properties do not have much impact on PCP bioavailability. (Supported by EPA grant CR827224).

671 TIME COURSE FOR TISSUE DISPOSITION OF DIMETHYLARSENIC ACID AFTER ACUTE ORAL ADMINISTRATION IN THE MOUSE.


USEPA, ORD, NHEERL, ETDA, Research Triangle Park, NC.

The primary metabolite excreted by most mammals after exposure to inorganic arsenic is dimethylarsenic acid (DMA(V)). Recent studies have shown that DMA(V) is a multi-organ tumor promoter in rodents and a bladder carcinogen in rats. This study examined the disposition of DMA(V) in tissues over time after acute oral administration. Adult female B6C3F1 mice (N=3-4/time point) were administered [14C]-DMA(V) orally at a dose level of 0.5 or 60 mg As/kg and sacrificed at selected time points (0.25-24 hr). Tissues were removed and analyzed for chemical-derived radioactivity (14C). DMA(V) was rapidly absorbed and distributed among the tissues, as evidenced by the detection of 14C in all tissues at the earliest time point (0.25 hr). Concentrations of 14C (Bq/mg g tissue) in the kidney, liver, and lung were greater than in blood at this time, indicating that DMA(V) is rapidly accumulated by tissue. The peak concentration of 14C in the tissues occurred between 0.5-2 hr after administration of DMA(V) and ranged from 0.4-2.6%. The tissues with the highest concentration (2.6%) were the lung for the low dose and the kidney for the high dose. There were no dose-dependent differences in peak tissue concentration of 14C. By 24 hr, the tissue concentration of 14C was considerably lower than observed at 0.25 hr. Approximately 0.01% of the dose was found in urine and less than 0.01% was found in other tissues. The concentration of 14C in these tissues was greater than in the blood at 24 hr. The data indicate that an acute oral dose of DMA(V) is rapidly absorbed, distributed, and excreted from the mouse. The rapid elimination of DMA(V) explains in part the low acute toxicity of this compound. (This abstract does not necessarily reflect EPA policy.)

672 THE POTENTIAL OF SELECTED CROP PROTECTION CHEMICALS TO ALTER HUMAN P-GLYCOPROTEIN ATPASE ACTIVITY.

L. G. Fox1, R. C. Peeler2 and L. T. Stavros2.

1 GENESTENT Corporation, Woburn, MA and 2 Syngenta Crop Protection, Inc., Greensboro, NC and 3 Wake Forest University, Winston-Salem, NC.

P-glycoprotein (Pgp, encoded by mdr1) is a member of the ABC transporter superfamily and is expressed in the capillary endothelial cells of the brain, gonads, placenta and other tissues. Pgp serves as a barrier to keep toxins out of the nervous system, gonads and fetus by actively pumping substrates across the cell membrane and out of the cytoplasm. ATP hydrolysis provides the energy for active transport. Numerous drug and crop protection chemicals (CPC) are substrates for PGP [Bain and LeBlanc, 1996, Toxicol. Appl. Pharmacol. 141, 288-290]. The potential of selected CPC to stimulate the ATPase activity of human PGP membrane (GENESTENT Catalog K228) was evaluated. CPC were incubated in a buffered solution containing CPC membranes and supplemented with MgATP, with and without sodium orthovanadate to inhibit PGP by trapping MgADP in the nucleotide binding site. Each CPC was assayed over a concentration range based on its solubility in DMSO to 100 µM in 96-well microtiter plates. The herbicides, atrazine, chlornofop-propargyl, did not stimulate human PGP ATPase activity in the concentration ranges tested nor did the insecticide, thiacloprid. The failure of these substances to alter ATPase suggests they are not substrates of PGP. The insecticide, emamectin-benzoate, suppresses the basal activity in the PGP membranes over the entire concentration range tested, indicating an inhibitory interaction with the PGP-ATPase. Additionally, when verapamil, an established substrate of PGP was co-incubated with increasing concentrations of emamectin-benzoate, the verapamil-stimulated ATPase activity was inhibited in a concentration-dependent manner. The trazine herbicide didekafluoromethyl, diminoclorotrimazine, as well as the fungicide, propiconazole, were stimulated activity in a concentration-dependent manner indicating that they are substrates for PGP. This screen further supports the premise that CPC, like pharmaceutical agents, may interact with PGP.

673 DISTRIBUTION OF CHLORPYRIFOS AND ITS PRINCIPAL METABOLITE TRICHLOROPYRIDINOL IN DAM AND NEONATE TISSUES/FLUIDS.


1 Battelle Toxicology Northwest, Richland, WA and 2 Battelle Memorial Institute, Columbus, OH and 3 NIEHS, Research Triangle Park, NC. (Supported by EPA grant CR827224).

Pregnant Sprague-Dawley rats were administered chlorpyrifos (CPF) via gavage (in corn oil) at doses of 0.5, 3.0, and 5.0 mg/kg gestational day 14 to postnatal day 7. CPF and its principal metabolite, trichloropyridinol (TCP), were assayed in the samples collected on postnatal day 7 from dams (blood, brain, liver and milk) and neonates (plasma, brains, and livers) at Battelle using gas chromatography-negative-ion chemical ionization mass spectrometry (GC-NCl/MS) methods. CPF was readily quantified in dam milk, liver, brain, and blood collected from dams administered 3 mg CPF/kg, but only detectable in neonatal plasma, liver, or brain from the 5 mg CPF/kg group. CPF accumulation in liver and detectable in brain samples collected from dams administered 3 mg CPF/kg. Generally, CPF concentrations in all study samples were not detectable in samples from either the control or 0.5 mg CPF/kg groups. In these tissues/fluids where CPF was observed, the concentrations increased with increasing CPF dose and the highest CPF concentrations were observed in dam milk. TCP was quantifiable in all tissues/fluids analyzed (blood, liver, brain, milk) from dams administered 0.5, 3.0, and 5.0 mg CPF/kg. TCP was detectable in plasma, liver, and brain collected.
674 KINETICS OF PERCHLORATE-INDUCED INHIBITION OF IODIDE UPTAKE IN TISSUES OF THE PREGNANT RAT AND FETUS.

AFRIHEST, Wright-Patterson Air Force Base, OH.

Perchlorate (ClO₄⁻) acts as a competitive inhibitor for iodide uptake into the thyroid gland. This results in perturbation of biosynthesis of thyroid hormones necessary for neurodevelopment in prenatal and neonatal periods. The objective of this project was to determine disposition of ClO₄⁻ in maternal and fetal tissues of Sprague-Dawley rats, and inhibition of iodide uptake in maternal thyroids. Pregnant rats (GD20, n=6) were intravenously injected with carrier-free ¹²⁵I (1.87 mg/kg, control) or with 1 mg/kg ClO₄⁻, then with carrier-free ¹²⁵I 24 h later. Dams were sacrificed at 0.5, 2, 4, 8, 12 and 24 h after injection. Blood, thyroid, skin, placenta, mammary gland, amniotic fluid, gastric tract (GT) and gastric contents (GC) were collected as well as fetal blood, skin and GT. Percent inhibition of iodide uptake in dam's thyroid was observed at all time points, the highest (over 85%) at 1 and 2 h, and the lowest (over 65%) at 0.5 and 24 h post dosing of iodide. Concentrations of iodide in control serum at 0.5, 1, 2, 4, 8, 12 and 24 h were 2.9, 2.5, 2.35, 1.84, 1.26, 0.98 and 0.36 ppm, respectively. Levels of iodide in amniotic fluid were lower than fetal skin levels except 24 h. Iodide levels of control dams' mammary gland at 4, 8, 12 and 24 h were 2.4, 2.19, 2.38 and 1.49 ppm, respectively, and these are higher than dam's serum levels. Sequestration of iodide was evident in both fetal skin and dam's mammary gland. This information was used to develop a physiologically-based pharmacokinetic model to illustrate target concentrations of ClO₄⁻ in prenatal period. In addition, this model will be employed to address potential risk associated with ClO₄⁻ exposure during gestation.

675 KINETICS OF PERCHLORATE AND IOIDE IN LACATING S-D RATS AND PUPS AT POSTNATAL DAY 10.

MonTech Environmental Technology, Inc., WPAFB, OH; AFRIHEST, WPAFB, OH and 1GeoCenters, Inc., WPAFB, OH.

Ammonium perchlorate (NH₄ClO₄), a groundwater contaminant resulting from military and industrial use, readily dissociates to NH₄⁺ and ClO₄⁻. The perchlorate ion (ClO₄⁻) competes with I⁺ for uptake into the thyroid, potentially causing hypothyroidism. Time course studies were performed to understand better the kinetics of ClO₄⁻ and iodide in lactating dams and nursing pups. At postnatal day (PND) 10 female Sprague-Dawley dams were intravenously (i.v.) injected with 0.1 mg/kg ClO₄⁻ in water and returned to their caged litters. Dam serum, thyroid, skin, mammary gland and gastric contents and pup serum, skin, gastric tract and contents were collected at 0.5, 1, 2, 4, 8, 12 and 12 h for ClO₄⁻ analysis. To investigate the inhibition of iodide uptake by ClO₄⁻, lactating dams at PND 10 were i.v. injected with 1 mg/kg ClO₄⁻ followed 2 h later with an i.v. injection of carrier free ¹²₅I and returned to their litters. Control sets of lactating dams were injected with carrier free ¹²₅I only and returned to their litters. Dam serum, thyroid, skin, mammary gland and gastric contents and pup serum, skin, gastric tract and contents were collected at 0.5, 1, 2, 4, 8, 12 and 24 h for ClO₄⁻ and iodide analysis. Perchlorate level in serum after dosing with 0.1 mg/kg ClO₄⁻ was 0.19 ± 0.02 mg/ml at 0.5 h and was not detectable at 8 h. Both male and female pups had 0.038 ± 0.003 mg/ml of ClO₄⁻ in serum at 12 h. Perchlorate exposure inhibited iodide uptake into thyroid of lactating dams. At 30 min the bound iodide level in dam thyroid after ClO₄⁻ exposure was 0.19 ± 0.02 ng/g whereas the level in dam thyroid after iodide only exposure was 0.08 ± 0.05 ng/g. The information generated from this kinetic study will support the development and validation of a ClO₄⁻ lactation model. A risk assessment for ClO₄⁻ supported by a lactation model will address the risks associated with ClO₄⁻ exposure in a sensitive population.

676 ETHYLENE GLYCOL KINETICS IN PREGNANT RATS: DIFFERENCES BETWEEN SLOW AND FAST DOSE-RATE EXPOSURES.

The Dow Chemical Company, Midland, MI and Pacific Northwest National Laboratories, Richland, WA.

Large bolus doses of ethylene glycol (EG) are developmentally toxic in rodents. However, human occupational and consumer exposures typically involve low doses and lower dose-rates (e.g., dermal, inhalation). This study examined the impact of dose-rate on maternal and embryonic kinetics of EG and its metabolites. Pregnant CD rats were dosed from gestation days (GD) 6-16 with 1000 or 2000 mg/kg/day of EG given either as a daily subcutaneous (SC) bolus injection (fast dose-rate) or continuous rate infusion (slow dose-rate) via an implanted pup. Rats were also dosed by oral gavage (fast dose-rate) on GD 11 at either 100 or 1000 mg/kg EG and its developmentally toxic metabolite, glycolic acid (GA) were determined in maternal blood samples on GD 7-13 in the SC bolus injection and continuous infusion study. Detailed analysis of the kinetics of EG, GA and exalid acid (OAA) were also determined in maternal blood, urine and kidneys, as well as conceptus fluids and embryos on GD 11-12 for the oral gavage and SC infusion study. EG levels were similar in maternal blood at maternal tissues and conceptuses within a given treatment group, while GA levels were consistently higher in tissues than maternal blood. Only levels at all dose levels were variable between similar to control levels in all samples regardless of dose. Blood and urine levels of GA and OAA were similar following the two administration routes. However, peak blood levels for both EG and GA were consistently higher (~3 fold for EG and ~5 fold for GA) following bolus oral and SC dosing than following constant rate SC infusion at comparable total dose levels. The lower levels of GA observed in the slow dose-rate group corresponded with the low toxicity characteristic of slow dose-rate administration of EG, as shown in previous studies. Therefore, bolus dosing with EG appears to significantly overestimate the risk of typical occupational and consumer exposures to EG.

Funded by the American Chemistry Council, Arlington, VA.
this study, 17C-BPA was administered via gavage at 10 mg BPA/kg body weight to timed-mated pregnant rats on gestation days 6, 14, 17, and to non-pregnant female rats. Blood samples were collected at selected time points over a 36-h period following oral administration. Plasma radioactivity, BPA and BPA-glucuronide were quantitated in pooled plasma samples by liquid scintillation counting or radiochemical profiling using HPLC. Pooled excreta samples from selected dose groups and collection intervals were profiled by HPLC to quantify total BPA parent, BPA-glucuronide and metabolites. Structural confirmation of BPA and BPA-glucuronide were confirmed, as in a previous study, for representative plasma, urine and fecal samples. Selected tissues were collected at sacrifice and analyzed for radioactivity, parent compound and metabolites, including blood, brain, embryo/fetus, fat, liver, kidneys, ovaries, placenta, uterus, skin, and remaining carcass. BPA pharmacokinet- ics and metabolism did not appear to be affected by pregnancy. There was evi- dence of enterohepatic circulation in all groups, based on a secondary peak in the plasma radioactivity concentration-time profile. Mean tissue concentrations were either non-quantifiable or <0.25 pg-equivalents/g tissue. No apparent selective affinity of either placenta or embryo/fetus for 17C-BPA-derived radioactivity was observed. Systemic exposure to parent was very low based on plasma BPA concentra- tions. BPA-monoglucuronide was found to be the major metabolite in both pregnant and non-pregnant rats. This study provides important evidence that the disposition of BPA is not altered by reproductive status. Sponsored by: PCB/BPA

Global Group, American Plastics Council, Arlington, VA.

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PHARMACOKINETICS OF 17C-GENISTEIN FOLLOWING I.V. ADMINISTRATION IN ADULT FEMALE SPRAGUE-DAWLEY RATS.

A. Upender, B. A. Elswick, H. D. Parkinson and S. L. Borghoff, CIT Centers For Health Research, Research Triangle Park, NC.

Genistein is a phytoestrogen present in soy food and virtually all rodent diets and has been reported to elicit detectable estrogenic effects in developing rodents. Understanding the placental transfer of genistein in rats at a possible critical time during development will be important for assessing potential effects. As a first step this study was designed to evaluate the clearance of genistein from blood of nonpregnant female Sprague-Dawley rats dosed with 17C-Genistein. A timed profile of genistein blood kinetics was established following intravenous administration of a high dose (40 mg/kg b.w) and low dose (4 mg/kg b.w) of 17C-Genistein (10 µCi/rat; 1 ml/kg, in 65% DMSO) 48 hours following jugular vein cannulation. Blood samples were taken at 0, 5, 15, 30, 45, 60, 90, 120, 180, and 240 minutes following dosing and total radioactivity in plasma by liquid scintillation spectrometry. Five minutes after the administration of genistein, the plasma concentration was 130 ± 28 and 7.9 ± 2.7 pg genistein equivalents/ml plasma following high and low dose, respectively. These plasmaw levels rapidly declined so that by one hour the levels were 16 ± 7.1 and 0.9 ± 0.2 µg genistein equivalents/ml in the high and low-dosed animals, respectively. Fitting the plasma concentration-time curves to a 2-compartment model the initial half-life (alpha-phase) was estimated to be ~12 min for the 40 mg/kg b.w. dose and ~7 min for the 4 mg/kg b.w. dose. The terminal half-life (beta-phase) derived from the curve of the high dose was approximately 2.3 h. Because the levels of genistein fluctuated during the terminal phase of elimination following the low dose, this half-life could not be deter- mined. These fluctuations may be influenced by enterohepatic circulation of genistein which is known to occur in rats. These data provide the kinetic information that can be used in conjunction with a PBPK model to estimate metabolic rate con- stants for genistein. This study was funded in part by the American Chemistry Council.

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DISTRIBUTION OF MANGANESE IN THE BRAIN OF STAINLESS STEEL WELDING FUME INHALED SPRAGUE-DAWLEY RATS.

J. L. Xu, J. Park, E. Park', S. Song', J. Han', Y. Chung' and H. Chung', Center for Occupational Toxicology, Korea Occupational Safety & Health Research Institute, Daejeon, Korea; and 'Department of Preventive Medicine, Chung-Ang University, Seoul, South Korea and 'Dep't of Pathology, Chung-Ang University, Seoul, South Korea.

Welders working in the confined space like shipbuilding industry have a risk of ex- posing a high concentration of welding fumes and of developing pneumocystosis or other welding fume exposure related diseases. Among them, manganese caused by welding fume exposure is still controversial and no clear demonstration of manganese movement into specific brain regions. To investigate distribution of man- ganese in the brain of welding fume exposed rats, welding fumes generated from manual metal arc stainless steel (MMA-30) were exposed to make Sprague-Dawley rats with concentrations of 63.6 ± 4.1 mg/m³ (low dose) and 107.1 ± 6.3 mg/m³ (high dose) total suspended particulates for 2 hrs per day, in an inhalation chamber for 90 days. Blood, brain, lung and liver were collected at the end of 1, 15, 30, 60, and 90 days of exposure, and the tissue samples were analyzed for manganese concentra- tions by atomic absorption spectrophotometer. Although there were dose dependent increases of manganese concentrations in the lungs of exposed rats during 90 days of exposure, only slight manganese increases were detected in the blood during exposure period. Major statistically significant increases of manganese concentra- tions in the brain were detected increased over 15-60 days of expos- ure. There were slight increases of manganese concentrations in the substantia nigra, basal ganglia (caudate nucleus, putamen and globus pallidus), temporal lobe and frontal lobe.

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CELL TYPE SPECIFIC DIFFERENCES IN FORMATE TOXICITY.

J. L. Emmrich, M. M. Henry, C. M. Smutsat, J. M. Burke and J. T. E. Ellis, Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI.

Methodol is an important health and environmental concern because of the neurotoxic actions of its metabolite, formic acid. Formic acid has been hypothesized to act as a mitochondrial toxin to produce retinal and optic nerve toxicity. Studies in our laboratory using a methylcholanthrene-senstive rodent model have revealed functional, meta- bolite and structural abnormalities in the retina consistent with this hypothesis. The present studies were undertaken to further define the mechanism of formate toxicity by examining cell-type specific differences and cellular consequences of formate toxicity. Madin-Darby canine kidney cells (MDCK), renal pigment epithelial cells (RPE), and a photoreceptor cell line (661W) were exposed to formate (1-1000mM) over 5 time courses (10 min, 24 hrs, and 13 days). Cellular formate concentrations, morphologic changes, and cellular ATP concentrations were assessed. Intracellular formate concentrations increased to a similar extent in all 3 cell types. In cultures exposed to 10 mM formate, concentrations increased from basal concentrations of 8.8 ± 1.1 μmoles formate/mg protein to a plateau concentration of 115.2 ± 12.5 μmoles formate/mg protein following 6 days of exposure. In cultures exposed to 100mM formate, intracellular formate concentrations increased for 6 days plateauing at 253 ± 21.4 μmoles formate/mg protein. Formate treatment produced mor- phologic changes in MDCK cells primarily characterized by cell swelling. No mor- phologic alterations were observed in RPE or 661W cells. Intracellular ATP concentrations were significantly decreased in MDCK cells and RPE cells within 24 hours. In 661W cells, ATP concentrations increased from 15 ± 2 to 26 ± 3 μmoles ATP/mg protein within minutes followed by a significant decrease in ATP concentrations to 5 ± 1 μmoles ATP/mg protein by 6 hrs. These data provide evidence of cell-type specific differences in the cytotoxic actions of formate. They further sug- gest that intracellular compensatory mechanisms are rapidly triggered following formate exposure. (RO1-E506648, RO1-EY1396 and P30-EY01931).

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ESTIMATING MINIMUM CRITERIA FOR JUDGING THE PERFORMANCE OF TOXICITY TESTS IN VALIDATION STUDIES.

J. W. Harbell, R. D. Crown and L. H. Boumer, 'Institute for In vitro Sciences, Inc, Catonsville, MD and Celltech Medical Evaluation Laboratories, Neudum, MA.

It is often stated that a new toxicity test (NTT) must provide a level of protection equivalent to or better than the reference test it will replace. It is less clear how to measure the performance of a reference test in a manner that is useful for comparison with a NTT. The purpose of this report is to describe an approach used to de- fine the predictive capacity of a commonly used in vitro method, the Draize eye irri- tation test. Data were obtained on the reproducibility of the Draize test from the scientific literature. These data were then used to define variability terms used in a computer simulation that models the predictive capacity of the Draize test. The va- lidity of the simulations was verified by comparing actual in vivo data with results from the simulations. The Draize test variability is greatest for the mildest irritants. The maximum expected correlation coefficient is 0.8-0.84. The expected sensitivity is 0.74-0.95, specificity 0.67-0.87, and accuracy 0.82-0.92 depending on the cutoff chosen. The width of the 95% prediction interval ranges between 16 and 93 for a predicted maximum average score of 55 on the 110-point scale. These performance statistics can be used as baseline criteria for judging the adequacy of NTT intended to replace the in vivo test.

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ESTIMATION OF DNA STRAND BREAKS IN LENS EPITHELIAL CELLS; WHITE LIGHT MEDIATED GENOTOXICITY.

N. P. Singh', P. E. Penn', W. R. Pondergrass' and N. S. Wolf', Bioengineering, University of Washington, Seattle, WA and 'Pathology, University of Washington, Seattle, WA.

It was hypothesized that white light induces DNA single-strand breaks/alkali labile sites in lens epithelial cells of 60 day old Brown Norway rats during the dissection process. Rats were narcotized using carbon dioxide gas and eye lenses were dissected
our either in white or red light at a distance of 3.5 inches and for a duration of approximately five minutes. Electrical input to lamps was adjusted such that the light delivered from each was approximately equal to the intensity of 100 foot candles (1076 lux). DNA single strand breaks (SSB) and alkali labile sites were estimated using the alkali microgel electrophoresis (COMET) assay. Dissection of lenses in live light induced significantly higher internal SSB (p < 0.0216), tail moment (p < 0.0048), tail length (p < 0.048), tail length (p < 0.048) as indices of DNA single strand breaks/alkali labile sites. In addition, we investigated the DNA damaging effect of visible light in a human lymphocyte model in which cells were exposed to white or red light. We evaluated the effect of red light for 15 minutes. Results of these experiments confirmed that visible light can induce DNA damage in individual, isolated cells. Our results on the ability of visible light to induce DNA single strand breaks/alkali labile sites may have numerous implications in both plant and animal cells. These findings may also relevant while conducting genotoxicity assays on cells in vitro.

684 VALIDATION OF A MODEL OF PHOTORECEPTOR DEGENERATION IN THE RAT.

A rat model of light-induced photoreceptor degeneration was validated in order to serve as an efficacy model for therapeutics directed towards prevention of retinal damage. The design included a positive control group which received an intravitreal injection of brain-derived neurotrophic factor (BDNF) in one eye and the negative control in the contralateral eye. A negative control group received the intravitreal injection of saline in one eye while the contralateral eye was an untreated control. Animals were housed under reduced illumination (20 foot candles) and 12 hour light cycle, for 10 days prior to injection. Twenty-four hours following the injections, the rats were placed into housing units which ensured continuous and complete (over a distribution) light exposure at approximately 200 foot candles, for 84 hours. Thereafter the animals were returned to standard caging, with a 12 hour light cycle, where they were maintained for 14 days. A series of stereotopic and photopic electrotinograms (ERGs) were performed prior to the injections and again at the end of the 16 day observation period. Following termination, 5 serial sections of each eye (from inferior to superior) including the outer nuclear layer were prepared and examined histopathologically. Both ERG and histopathology examinations confirmed the light exposure to have induced photoreceptor degeneration in negative control and untreated eyes. Anticipated neuroprotective action of the positive control compared to the negative control was also evident and therefore the model was considered suitable for use in efficacy evaluations of neuroprotective agents in retinal indications.

685 ROLE OF LENTICULAR CHOLESTEROL BIOSYNTHESIS AND SMALL GTP-BINDING PROTEINS IN THE CJ-12, 918 RAT CATARACT.
D. J. Baltnikov, S. Paletti, S. M. Kajji, M. D. Algo and M. E. Verdugo.

1 Drug Safety Evaluation, Pfizer Global R&D, Groton, CT and 2 Cancer Biology, Pfizer Global R&D, Groton, CT.

Purpose: Alterations in cholesterol biosynthesis, either through drug inhibition (i.e., lovastatin or tiloprad) or inherent genetic defects, are associated with cataract formation in animals and humans. We have previously reported CJ-12, 918 inhibits lenticular cholesterol biosynthesis before significant alterations in lentilucal AT1, GSH, and crystallin content, or clinical evidence of cataract formation in the rat. Rao et al. have demonstrated in vitro thatLovastatin inhibits the incorporation of small GTP binding proteins in lens epithelial cells (LEC) and have proposed this as the main mechanism of the lovastatin in vitro and in vivo cataractogenesis. The following study investigated whether CJ-12, 918, likeLovastatin, alters small GTP binding protein (GTPase) content in primary cultures in vitro. Methods: Rat and canine LEC were treated with CJ-12, 918 (10-50 μM) or Lovastatin (10-50 μM) for 48 hrs. RhoA/RhoB and Rabs incorporation was analyzed in all the membrane fractions, and phosphorylated ERK1/2 in the whole cell lysate by Western blot. Results: No change in rat or canine LEC GTPase patterns was observed with CJ-12, 918, treatment. However, treatment with Lovastatin decreased Rho A and Rho expression in the membrane fractions with a concurrent increase in the cytoplasm and decreased phosphorylated ERK1/2 in the whole cell lysate. In the canine LEC, these observations changes with Lovastatin treatment were concentration dependent. The effects of Lovastatin in treatment (50 μM) in the rat LEC were alleviated with mevalonic acid supplementation (100 μM). Conclusions: Our data supportLovastatin inhibition of small GTP binding protein supplementation in LEC in vivo. CJ-12, 918 does not inhibit small GTP binding protein suppression. The major mechanism of cj-12, 918 cataract appears to be inhibition of lenticular cholesterol biosynthesis late in the pathway.

686 OCULAR IRRITATION OF COMMON CONSUMER PRODUCTS USING THE CULTIVATED BUNGE LENS.

In recent years, abattoir-supplied bovine lenses have been cultured to measure ocular irritation as an alternative to the Draize Test. This approach uses cultured cells and a laser-scanning instrument to measure the optical quality of bovine lenses exposed to potentially harmful agents. Quantitative results are obtained rapidly and objectively, and with greater sensitivity than the Draize Test or other suggested alternatives such as BCOPI. We report here the results of a study, which demonstrates the ability of the cultured bovine lenses to detect ocular irritation caused by common semi-solid and solid materials that are partially irritating. The results of this study are valid for up to 90% of the final concentration of the test material.

687 INTERIM DATA FOR A NINE-MONTH LOCAL TOLERANCE AND TOXICITY STUDY OF EYE001 GIVEN BY INTRA-VITREOUS INJECTION TO BEAGLE DOGS.

1 Calvert Preclinical Services, Inc., Oliphant, PA, W. D. Associate, Marion Island, FL, and 2 EYETech Pharmaceuticals, New York, NY.

EYE001 is an RNA oligonucleotide attached to a PEG group, inhibits angiogenesis in the retina of the eyes of animals. The objective of this study was to determine the local tolerance and toxicity of EYE001 injected into the vitreous humour of seven beagle dogs for a period of nine months. Animals received intra-vitreal injections of vehicle or EYE001 (0.1 ml volume at 3.0 ng/ml, 10.0 mg/ml or 30.0 mg/ml) into each eye. Dosage was calculated on the apatam (RNA) component of the test article. While animals were under light anesthesia, an eye was injected to keep the eyelid open. The needle tip was inserted perpendicularly to the globe at a position 7 mm superior from the limbus on the scleral portion of the globe and pressure applied until the needle tip just penetrated the sclera. The angle to the orbital was maintained in a superior inferior fashion. The needle tip was then inserted retrograde to a point that facilitated administration of the test article to the posterior portion of the eye. Positive toxicity was observed in the posterior chamber using an indirect ophthalmoscope and slit lamp, fundus photography, electrotinographic and intracranial pressure measurements, daily macroscopic observation, clinical signs, body weight and food consumption determinations. Animals had blood and urine samples collected for hematologic, coagulation and serum clinical chemistry profiles and semi-quantitative urinalysis prior to study initiation and at weeks 13 and 27. Through thirty weeks, the data indicate that EYE001 does not appear to cause local or systemic toxicity in the beagle dog.

688 PROTECTIVE ROLE OF THE HUMAN CORNEAL ALDH3A1 AGAINST UV-INDUCED TOXICITY.
V. Vasiliev, R. Manzer and A. Pappa. Department of Pharmaceutical Science, University of Colorado Health Sciences Center, Denver, CO.

Aldehyde dehydrogenase 3A1 (ALDH3A1) is a significant constituent of mammalian corneal epithelium, representing 20-40% of total corneal water soluble protein. Several hypotheses are proposed for the role of ALDH3A1, which include (a) metabolism of cyotoxic aldehydes formed during UV-induced lipid peroxidation, such as 4-hydroxynonenal (HNE), (b) direct absorbance of UV-light, and (c) generation of NAD(P)H, which has recently been assigned a UBV absorbing species. The aim of this study was to develop human corneal epithelial cell lines expressing the human ALDH3A1 at levels similar to those observed in vivo, and to

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study the role of this enzyme against UV-induced toxicity. We have established three types of transfected cell lines expressing ALDH3A1 at high, moderate and low levels. Our data indicate that ALDH3A1 protects against 4-HNE-induced cytotoxicity. Furthermore, Western blot analysis using antibodies against 4-HNE-activated Friedlander revealed that ALDH3A1 also prevents activation of the cornal epithelial proteins by 4-HNE. Enzyme kinetics with a human recombinant ALDH3A1 showed a Km of 32 mM for 4-HNE. Finally, ALDH3A1 expression protected cornal epithelial cells against UVB and UVC-induced cytotoxicity. In conclusion, the human ALDH3A1 appears to be a significant factor against UV-induced toxicity. [Supported by NEI 1 R29 EY11490].

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INDUCIBLE EXPRESSION OF GLUTAMATE-CYSTEINE LIGASE AFFECTS CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN TRANSGENIC MICE.

S. Shi, D. Bouali, C. C. White, M. J. Dabrowiski, S. L. Sirnuovanachan, F. M. Farin, R. M. Pierce, C. B. Wrenn, W. C. Ladiges, N. Fausto, S. Y. Tsai, B. W. O'Malley and T. J. Kavanagh. Environmental Health, University of Washington, Seattle, WA; Comparative Medicine, University of Washington, Seattle, WA; Pathology, University of Washington, Seattle, WA; Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY; and Molecular and Cell Biology, Baylor University College of Medicine, Houston, TX.

Glutathione (GSH) is a major free radical scavenger and is important in detoxification of xenobiotics such as carbon tetrachloride (CCL4). The rate-limiting enzyme in GSH synthesis is glutamate-cysteine ligase (GCL), which is composed of catalytic (GCLc) and modifying (GCLm) subunits. Transgenic mice that conditionally over-express GCLc and/or GCLm using a mifepristone (RU486)-sensitive GenSwitchTM system have been generated, and we have demonstrated RU486-induced enzyme activity and transgene(s) in the liver of these animals. We examined the influence of GCLc and GCLm over-expression on the extent of CCl4-induced liver toxicity. GCL transgenic mice were less susceptible to liver injury following RU486 administration and subsequent CCl4 treatment as revealed by serum ALT activity measurements and histopathology scores of the liver. GCL transgenic mice also showed lower levels of lipid peroxidation that is an early event in CCl4-induced liver injury. In contrast, administration of RU486 in nontransgenic mice synergized carbon tetrachloride-induced liver injury.

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REGULATION OF THE MOUSE GLUTAMATE CYSTEINE LIGASE MODIFIER SUBUNIT GENES VIA THE ARE AND NRF2/API BINDING SITES.

P.N. Hudson, F. Be, and T.J. Kavanagh. Environmental Health, University of Washington, Seattle, WA; and Pathology, University of Washington, Seattle, WA.

We previously reported that basal and BHQ-inducible expression of the mouse glutamate-cysteine ligase modifier subunit gene (Gclm) was dependent upon regulatory elements located at 788 bp upstream of its transcription start codon. This region is highly homologous to the human GCLM promoter and contains a conserved antioxidant response element (ARE), Electromobility shift assays (EMSA) reveal constitutive and BHQ-inducible binding of mouse Hepa-1 nuclear factors to this ARE, and mutagenesis of the ARE in a luciferase reporter construct containing 13 kb of Gclm promoter preferentially diminished inducible expression and affected basal expression as well. EMSA reveals constitutive and BHQ-inducible binding of nuclear factors at a putative Nrf2/Api site 90 bp upstream of the ARE. A single base-pair substitution at this site reduced basal expression of the reporter 100-fold while BHQ induction was conserved. The transcription factor Nrf2 mediates Gclm expression as evidenced by a 10-fold reduction in luciferase reporter activity in Nrf2-deficient cells which can be partially restored upon cotransfection with Nrf2 cDNA. Deletion constructs indicate that Nrf2-responsive elements lie within the region containing the ARE and upstream Nrf2/Api site, and inclusion of a Nrf2 or a Nrf2/Nrf2 antibodies inhibits binding and induces a subdued bandshift respectively in binding reactions for both the ARE and Nrf2/Api sites. Finally, mutations of the ARE in reporter constructs abolished basal and inducible expression in wild-type MEFs but had no effect Nrf2-deficient cells. Taken together, these data indicate regulation of Gclm by the transcription factors Nrf2 and Nrf2 via the ARE and upstream Nrf2/Api binding site.

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GLUTAMATE CYSTEINE LIGASE LEVELS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTE SUBTYPES.


Glutathione (GSH) plays a role in the immune response associated with inflammation. GSH dismutation has been implicated in inflammatory lung diseases such as idiopathic pulmonary fibrosis, cystic fibrosis and chronic beryllium disease. Glutamate cysteine ligase (GCL) is the enzyme catalyzing the rate-limiting step in the formation of GSH. The aim of this investigation was to determine the levels of the GCL catalytic subunit (GCLC) in human peripheral blood lymphocytes (PBPL) subtypes according to cell cycle in normal healthy individuals. PBPL glutathione (GSH) and glutathione (GSSG) levels were measured by a rabbit polyclonal anti-GCLC peptide antibody and a goat anti-rabbit FITC labeled secondary antibody. PBPL subtypes and cell cycle phases were determined by staining for lymphocyte surface markers and DNA content. Antibody binding was then analyzed on a flow cytometer and a multiparameter analysis performed utilizing Multiplo software. PHA stimulated lymphocytes were found to have similar GCLC levels as non-stimulated lymphocytes. CD8 T-cells have the highest levels of GCLC. CD4 monocytes have the lowest GCLC levels which decrease further upon PHA stimulation. CD19 PHA stimulated B-cells have intermediate levels of GCLC which increase upon PHA stimulation. Cell cycle analyses suggest G1 phase GCLC levels are highest in CD4 and CD8 T-cells. Whereas, G1 and S/G2 phase GCLC levels are similar in CD14 monocytes. G1 phase GCLC levels in CD14 T-cells are higher in the PHA stimulated B-cells than in the non-stimulated B-cells. This information will contribute to the understanding of the role of GSH and GCL in the proliferation of lymphocyte subtypes and in defining potential immunotoxicity within sensitive populations. This study was supported by the Department of Energy via the Consortium for Risk Evaluation with Stakeholder Participation and NIH grants P30-ES04695, P30-ES7033.

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GENERATION OF A Gclc(-/-) INDUCIBLE KNOCKOUT MOUSE LINE, USING THE Cre/loxP SYSTEM.

Y. Yang, T.P. Dalton, L. Dricot, Y. Chen, H.G. Shepherd, and D.W. Nebert. Center for Environmental Genetics, and Department of Environmental Health, University of Cincinnati Medical Center. Cincinnati, OH.

Glutathione (GSH) is the most abundant nonprotein thiol in mammalian cells. Glutamate-cysteine ligase (GCL) is the rate-limiting enzyme in the glutathione (GSH) biosynthesis pathway. This enzyme is a heterodimer comprising a catalytic subunit (GCLc) and a modifying subunit (GCLm). Although Gclc(-/-) mice homozygous for the targeted disruption of Gclc have provided insight into the developmental roles of GSH synthesis, the early embryonic lethality of these animals precludes studies of GSH during later development and organogenesis. To overcome this obstacle, we have generated an inducible knockout mouse line, where Gclc gene was flanked by loxP sites in introns 3 and 6. Homozygous Gclc(-/-) mice containing the flanked Gclc gene have the GCLc level as Gclc(+/-) wild-type mice, suggesting that insertion of the loxP sites is not critical. The ability of the Cre recombinase enzyme to cause deletion of the floxed allele was tested in situ by crossing Gclc(-/-) mice to mice carrying a Cre transgene. Mice of both deletion of the floxed allele in the Creloxp progeny resulted in embryonic lethality that coincided with the phenotype observed in the conventional knockout mouse. This inducible knockout mouse line will serve as a valuable tool to investigate the role of GSH synthesis in a tissue- and temporal-specific manner. Supported, in part, by NIH grants P30-ES06096 and ROI AG02325.

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GLUTATHIONE S-TRANSFERASE EXPRESSION IN HUMAN LIVER MITOCHONDRIA.

J.L. Gardner, D.S. Barber, and R.P. Gallagher. The Biological Sciences, University of Florida, Gainesville, FL.

The mitochondrial environment is rich in reactive oxygen species (ROS) that may ultimately participate in membrane proteins and generate highly reactive unsaturated aldehydes such as 4-hydroxy-2-nonenal (4HNE). Accordingly, multiple protective pathways have arisen to reduce mitochondrial dysfunction during oxidative stress. In the present study, affinity chromatography was used to isolate glutathione S-transferases (GSTs) from human hepatic mitochondria. HPLC-subunit analysis of affinity-purified liver mitochondria suggested the presence of at least two or more mitochondrial glutathione S-transferases (GSTs). Amino acid sequencing and immunological analysis of affinity-purified liver mitochondria and hepatocytes suggested the presence of two alpha class GSTs related to glsTA1A and gstA1A2 enzymes that protect against oxidative injury. Matrix-assisted laser desorption/ionization mass spectroscopy was used to further characterize the mitochondrial GST isoforms. Results from this ongoing investigation suggest that GST may represent an important ingredient to protect the mitochondria from the deleterious effects of ROS. Supported by N.I.H. ES-09427 and U.S.E.P.A. R-82474.

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OVEREXPRESSION OF A MITOCHONDRIAL GLUTATHIONE TRANSPORTER IN NRK-52E CELLS.

D.A. Priti, L.H. Ladd, and L.H. Matherly. Pharmacology, Wayne State University School of Medicine, Detroit, MI; and Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI.

The dicarboxylate carrier (DCC) of the mitochondrial inner membrane was cloned from total rat kidney RNA, expressed in E. coli as a His-tagged protein, and shown to transport both malonate and glutathione (GSH) with properties that
were similar to what we described previously. The cloned, expressed, purified and
reconstituted DCC protein exhibited saturable uptake of both maltose and GSH. Uptake of
GSH was inhibited by several competitive substrates (b-maltulose, L-
D-galactosyl-1-D-galactoside, ethylamine, maltose) and by pyruvate 5-phosphate
and mersalol. Maltose uptake was inhibited by b-maltulose, pyruvate,
GSH. Like many mammalian 5-phosphate, mersalol and, most significantly, GSH. We conclude that many mitochondrial processes are, therefore,
down-regulated. NRK-52E cells transfected with the cDNA for rat
DCC-overexpressed mitochondrial GSH transport activity. Expression of the rat
DCC-Hc1c fusion protein was confirmed by Western blot analysis. Mitochondria
from transfected cells exhibited 2.1 to 6-fold higher activity for uptake of 5 mM
GSH and 2.0 to 10-fold higher activity for uptake of 1 mM maltose as
compared to mitochondria from normal cells. We are currently developing stable
transfected that overexpress the DCC protein and investigating the effect of higher mi-
tochondrial GSH transport activity in the transfected cells on susceptibility to
chemically induced apoptosis. Preliminary results suggest that transfectants are
indeed less sensitive than normal cells to chemically induced injury. (Supported by
NIDDK Grant DK45725.)

695 ALKYLATION AND INACTIVATION OF HUMAN GLUTATHIONE TRANSFERASE ZETA (GSTT1-1) BY MALELYCTONE AND FUMARLYCTONE.
H. B. Lasturne, D. G. Luebber, P. G. Board and M. W. Anderson. University of
Rochester, NY, University of Arizona, Tucson, AZ and Australian National University, Canberra, Australia.
Glutathione transferase zeta (GSTT1-1) catalyzes the cis-trans isomerization of ma-
lylactoacetate (MAA) or malelyctone (MA) to fumarylacteate or fumaryl-
actone (FA), respectively. GSTT1 also catalyzes the glutathione (GSH)-depend-
ent biotransformation of a variety of x-halohydrins, including dichloroacetate acid
(DCA). This study was designed to investigate the mechanism of inactivation of
GSTT1-1 by MA and FA and to determine the covalent modification of
GSTT1-1 by MA and FA in the presence and absence of GSH. MA and FA (0.01-
1 mM) inactivated GSTT1-1 in a concentration- and time-dependent manner and
these effects were blocked by GSH. MA or FA did not, however, inactivate the
C64a mutant of GSTT1-1. ESI-MS/MS and SALSA (Scoring Algorithm for
Spectral Analysis) analyses of tryptic digests of GSTT1-1 variants revealed that the
active site (SSC'5WR) and C-terinal (L158,EAVQYCHPR) cysteine residues
of GSTT1-1 were modified by MA and FA. GSTT1-1 (NH2
HOCOCH=CHOCOCH3) addition caused 156 Da shifts in the masses of the modified peptide ions and in their MS-MS fragment ions. Alkylation of the active site cysteine residues, but not of the C-terminal cysteine,
was relatively less intense when GSTT1-1 variants were incubated with MA or FA in
the presence of 3-methyl glutathione. These data indicate that MA and FA are
substrates and product inhibitors of GSTT1-1 and covalently modify GSTT1-1 at
the active site cysteine residue in the absence of GSH. That these effects are blocked
by GSH. NRK-52E cells transfected with normal GSTT1-1 from normal cells.
Alkylation of the active site cysteine residues, and that in the C-terminal cysteine,
were relatively less intense when GSTT1-1 variants were incubated with MA or FA in
the presence of 3-methyl glutathione. These data indicate that MA and FA are
substrate and product inhibitors of GSTT1-1 and covalently modify GSTT1-1 at
the active site cysteine residue in the absence of GSH. That these effects are blocked
by GSH.

696 DEPENDENCE OF CYTOLOGICAL GRADINGS OF EXFOLIATED UROTHELIAL CELLS UPON GSTM1 AND GSTT1 POLYMORPHISM IN FORMERLY BENZIDINE-
EXPOSED WORKERS.
Q. J. Ma, G. F. Lin, J. C. Chen, J. H. Shen, C. Q. Xiang, K. Gelles and D. S. Zhang. Sino-German Laboratory of Toxicology, Shanghai Institutes of Biological Sciences, Shanghai, China. Municipal Center for Disease Prevention and Control, Shanghai, China and Institute of Occupational Physiology at the University of Dortmund, Dortmund, Germany.
The distribution of the polymorphic alleles of the genes coding for glutathione-S-
transferases (GSTs) M1 and T1 was compared to results of a cytological grading according
to Papamolyptil (of exfoliated urothelial cells in a non-diseased-high risk group
formerly exposed to benzidine in the Shanghai dyestuff industry (n=317). All
subjects were genotyped for GSTT1 and M1 gene polymorphism by allele-specific
PCR. Individuals were stratified according to their smoking status, occupational
exposure, the number of cigarettes smoked per day and duration of exposure.
A subgroup of 78 cases with cytological gradings grade III or higher (according to
Papamolyptil) showed a significant under-representation of the combination of
GSTM1 00 and M1 00 genotypes compared to 238 subjects with a cytological
classification lower than grade III (OR=0.55, 95% CI=0.31-0.98, P=0.04). These
results suggest that neither the GSTM1 00 or GSTT1 00 genotypes alone nor
the GSTM1*00/GSTT1*00 combination do have a clear association with cytological changes in exfoli-
ated urothelial cells from individuals previously exposed to benzidine in
Shanghai. This is contradictory to studies reporting that the GSTM1 00 genotype
in Shanghai is associated with an increased risk for bladder cancer in the general population,
mostly outside China.

697 CELLULAR GLUTATHIONE DISTRIBUTION DURING OXIDATIVE STRESS
New York State Department of Health, Albany, NY.
Oxidative stress causes molecular changes within cells that can lead to cytokine
overproduction. Glutathione, a ubiquitous thioredoxin tripeptide, plays a major role in
regulating these effects on oxidant. Glutathione in its reduced form (GSH) is also
assumed to assist in intracellular protein translation and trafficking. With the use of
a monoclonal antibody (mouse lgG; 8.1 GSH; Stress, Genec) against the
mitochondria adduct with N-ethylmaleimide, we determined the cellular
distribution of GSH in three human leucocytes cell lines (Jurkat, T cells; Daudi, B cells
and THP-1, monocyte-like) by an immunoelectron microscopy (gold conjugated
anti-mouse lgG). Immunoelectron microscopy was used in this study. The
mitochondria were the major target for detection. In general, the immunoelectron
microscopy results were similar to results by flow cytometry with regard to the amount of
GSH in the various cell lines after the oxidative stresses. Work support.
In part, by NIH grant ES03778.

698 NUCLEAR GLUTATHIONE REDUCTASE ACTIVITIES ARE INCREASED DURING PHASE OF CELL CYCLE IN CHO CELLS.
L. K. Rogers, T. N. Hansen, S. E. Welty and C. V. Smith. Pediatrics, Children's
Research Institute, Children's Hospital, The Ohio State University, Columbus, OH.
Glutathione reductase (GR) is a key enzyme in the glutathione antioxidative
pathway, reducing glutathione disulfide (GSSG) to the thiol form (GSH).
Cells have previously demonstrated GR localization in the microtubule,
the nucleus and the nucleoli of rat liver tissue. The goal of the present study was to test the hypothesis that GSH activities increased in cell cycle-dependent manner, which would
suggest a role for GR in regulation of cell cycle progression and related cell
responses. CHO (Chinese Hamster Ovary) cells were plated at 10,000 cells/ml in
the presence of 3-methyl glutathione. In the results of the experiments allowing the cells to arrest in G0. At 6 h, the CHO cells were
re-fed CHO media with 10% FCS and were harvested at 0, 4, 16, and 20 h.
Cells from each time point were harvested for subcellular fractionation using
the digitonin solubilization method of Bronfman (Anal Biochem 255:252), and nuclei
and mitochondria were separated by centrifugation. Additional cells were fixed
with ethanol and stained subsequently with propidium iodide for flow cytometric
analysis, to determine the degree of synchrony and the stage of cell cycle. Flow cy-

699 EFFECTS OF FASTING ON TISSUE CONTENTS OF COENZYME A AND RELATED INTERMEDIATES.
S. E. Welty, F. A. Jennishena, K. S. Joppert-Davis, L. C. Watters, L. K. Rogers
and C. V. Smith. Pediatrics, Children's Research Institute, Columbus, OH.
Exposure of rats and mice to hypoxia decreases lung content of the (CoASH) con-
tents, with a decrease of 50% observed in adult male Fischer 344 rats exposed to
>95% O2 for 48 h. The decreases in lung CoASH levels are not accompanied by

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increases in contents of the mixed disulfide with GSH (CoASSG), as might be expected of a primary oxidative stress on GSH status. Animals exposed to hyperoxia exhibited decreased food intake, and the present studies were to test the hypothesis that fasting would decrease lung CoASH contents. In addition, suggesting a mechanism for the effects of hyperoxia, adult male Fischer-344 rats were examined after 0, 24, or 48 h of fasting (n=5, 5, 6, respectively). Fasting for 24 or 48 h did not affect lung CoASH levels or weights, despite 6 and 12% losses in body weights. Lung GSH concentrations (nmol/g tissue) and contents (nmol/wet organ) and GSSG contents were lower in rats fasted for 48 h than in fed rats. Liver weights and GSH and GSSG contents and concentrations were lower in rats fasted for 24 or 48 h than in fed rats. Hepatic CoASH concentrations increased during fasting, but hepatic contents of CoASH remained remarkably constant. Liver protein concentrations (mg/wet organ) decreased after 24 and 48 h, but protein concentrations (mg/g tissue) were higher in rats fasted 48 h than in fed rats. Overall, GSH, GSSG, and protein contents in liver and skeletal muscle decreased with fasting, but significant changes in CoASH levels were not observed. Diminished food intake in animals exposed to hyperoxia may contribute to effects on GSH and GSSG contents, but does not explain the effects of hyperoxia on lung CoASH contents. CoASH and derived thiocyanate participate in many cellular functions, and if CoASH depletion proves to be relevant to lung injury caused by hyperoxia, dietary supplementation or support of mechanisms needed to sustain CoA levels could be helpful in preterminally born infants as well as in adults.

700 THE NATURE OF PROTEIN DISULFIDE ISOMERASE: ALKYLATION BY THE EPISULFINIUM ION DERIVED FROM S-(2-CHLOROETHYL)GLUTATHIONE.

R. S. Kasel and D. J. Reis. Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR; and Biochemistry and Biophysics, Oregon State University, Corvallis, OR.

Glutathione (GSH) protects cells although GSH conjugation can cause cytotoxicity by enhancing the reactivity of some xenobiotic chemicals, i.e., halogenated alkanes, quinones, and isothiocyanates. GSH conjugation to form S-(2-chloroethyl)glutathione (CEG) as a consequence of 1, 2-dichloroethane (DCE) exposure is another example of a toxicant reaction. CEG can form an electrophilic epiposulfinium ion that can react with specific sites in DNA or proteins. Evidence indicates that mutagenicity of DCE is due to the GSH conjugate. Our experiments are based on the reactivity of this episulfinium ion with proteins of the thioredoxin family (i.e., thioredoxin (TRX) and protein disulfide isomerase (PDI). Mammalian PDI has two thiodiol fold active site in each containing two cysteines. When reduced, these four cysteines are expected to be the major targets of alkylolation by the episulfinium ion of CEGs. Preliminary data comparing reactivity of active site sequence if PDI reactivity follows the same pattern observed with TRX. Reduced recombinant PDI was incubated for 90 minutes with a range of CEG concentrations from equimolar to 50-fold excess CEG. Based on ESI-MS and MALDI-MS of the whole protein before and after alkylation, 90% of the protein is either monoaalkylated (38%) or bisalkylated (12%). Further increasing CEG concentrations shows a rapid increase in native PDI to non-detectable amounts in 50 fold CEG excess. Alkylation of PDI does not interfere with an inhibition of redutcating or isoreductase activity as well as the alkylolation of TRX. Lactate dehydrogenase carbamylation of PDI inhibits activity suggesting that the CEG derived episulfinium ion may not as accessible to the active site PDI as is observed with TRX. Peptide mapping of alkylated PDI and further sequencing of modified peptides by ESI-MS/MS is underway. (This work was supported by grants from the NIEHS (ES00040 and ES00210).)

701 POSTEXPOSURE TREATMENT WITH 2-MERCAPTOPETHANE SULFONIC ACID (MESNA) INCREASES THE SURVIVAL OF MICE FOLLOWING EXPOSURE TO PHOSGENE.

S. L. Easkin and A. M. Scinto. Pharmacology, USAMRICD, Aberdeen Proving Ground, MD.

Phosgene (CG) is a highly reactive oxidizing agent used industrially as a chemical intermediate for the production of pharmaceuticals, pesticides, and polyvinyl rubber products. CG can cause life-threatening pulmonary edema by reacting with peripheral lung compartment tissue components. The insidiousness of CG inhalation is that there is a clinical lung phase where edema is not usually evident until 6-36 hours after exposure. Treatment modalities that have been currently relied on pretrement. This study was designed to investigate the effects of a clinically available sulfhydryl donor, MESNA. Postexposure survival rates and pulmonary edema formation (% of mice) after exposure to 20 mg/m3 (6ppm) CG for 20 min followed by room air washout for 5 min. Four groups of 18 mice each were injected i.p. at 20 min and 3 hr after exposure with 0 (saline), 5, 25, or 50 mg/kg MESNA. Within 12 hr of exposure photometric analysis was performed on lung tissue dry weight (DW), wet weight (W), and dry/wet weight ratio (W/D). Survival rates (SR) were also determined at both 12 and 24 hr postexposure. Both 5 and 25 mg/kg MESNA had no positive effect on any parameter measured. However, at 12 hr the 50 mg/kg dose showed a marginal decrease in lung tissue W/D compared with 0 mg/kg, 1.16±0.10 mg vs 1.35±0.09 mg (P<0.07), respectively. MESNA, 50 mg/kg, also significantly decreased the W/D ratio, 7.2±0.5 vs 6.0 mg/kg, 1.1±0.038 also at 12 hr. SR for the 50 mg/kg mice was 72% at 12 hr, P<0.01 (chi square) compared with 44% for 0 mg/kg. These results significantly enhanced lung tissue protective nonprotein sulphydryl levels. 21±2 umol/kg vs 0 (0.0001), 14±1 mg/kg MESNA, 2.9±0.1 (P<0.01) and reduced lung tissue protein oxidation, 5.2±1 vs 14.8±1 umol/kg MESNA (P=0.0001), at 12 hr. We conclude that the sulfhydryl donor MESNA, may be useful as a therapeutic agent against phosgene-induced acute lung injury by ameliorating PEF and reducing tissue oxidation.

702 ACROLEIN DEPLETES GLUTATHIONE AND THIOREDOSIN IN HUMAN BRONCHIOLAR CANCER CELLS.

J. L. Kao and J. L. Kohler. Dept. of Pharmacology and Toxicology College of Pharmacy, The University of Texas at Austin, Austin, TX.

Gluthionone (GSH) and thioderoxin (Trx) are important components of the cellular thiol redox system. Both play critical roles in regulating the activity of various transcription factors such as NF-kB and AP-1 that are involved in cell growth and cell death. Acrolein is a highly reactive, β-unsaturated aldehyde commonly found in the environment, particularly as a component of smoke. Acrolein's toxicity is linked to its electrophilic nature, and appears to correlate with GSH depletion. However, the effects of acrolein on other cellular thios is less well known. Recent data demonstrate with bovine maleiside detection of free sulphydryl on PVDF membranes that, beginning 1 hour after treatment of H272 human bronchiolar cancer cell lines with 100nmol of acrolein or more, there is an overall decrease in protein thios. In addition, treatment H272 cells for 0.5 h with the same doses of acrolein depletes GSH levels in a dose-dependent manner and causes a loss in Trx protein levels. The thiol oxidant diacrine also caused a loss in Trx protein. These acrolein-mediated changes in GSH and Trx are likely to disrupt the activity of various transcription factors and, in conjunction with an inhibition of caspase activities, may be important in the necrotic (apoptotic) cell death caused by this aldehyde. (Supported by ROI ES07971 and Center Grant ES07844.)

Western blot of thiorodoxin using 50 pg of a H272 cell lysate. Treatments were for 0.5 h.

<table>
<thead>
<tr>
<th>Acrolein (nmol/cell)</th>
<th>200</th>
<th>400</th>
<th>800</th>
<th>Control</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamide (mM)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
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</tr>
</tbody>
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703 EFFECT OF 2, 4-HExADION ON TISSUE GLUTATHIONE AND MALONDIALDEHYDE LEVELS IN FISCHER 344 RATS.


2, 4-Hexadion (2, 4-HX) is an α, β-unsaturated aldehyde formed by peroxidation of unsaturated lipids that causes neoplasms in the forestomach of rats. The objective of this study was to investigate the mechanism of action of this agent. 2, 4-HX was administered orally in corn oil to male and female Fischer 344 rats for 10 days. Plasma, liver, and forestomach samples were collected and analyzed for malondialdehyde (MDA) and glutathione (GSSG and GSH) by HPLC with fluorescence detection. No mortality, clinical signs or body weight changes were noted in rats administered either 90 or 120 mg/kg/day 2, 4-HX. Marked changes in the forestomach were observed microscopically including chronic inflammation, anacrosis, hyperkeratosis, ulceration and squamous epithelial cell papillomas; the severity was dose dependent. Significant differences in MDA content of plasma, liver or forestomach were not observed in the treatment groups compared to the vehicle or untreated groups, except for plasma MDA, which was slightly decreased in rats treated with 120 mg/kg/day. There was no apparent effect of either dose of 2, 4-HX on plasma levels of GSH. In the liver, the total amount of GSH was decreased with increasing dose of 2, 4-HX at 1 and 4 hr post-dose, but by 24 hr post-dose the total GSH levels returned to those observed in the vehicle controls. In contrast, 2, 4-HX administration increased the total amount of GSH in the forestomach, and at 1 hr and 4 hr post-dose this was largely due to increased GSSG; by 24 hr forestomach GSH levels had also been elevated. The GSH response at 24 hr was decreased by administration of 2, 4-HX treatment. In summary, 2, 4-HX treatment
for 10 days alters the tissue glutathione levels in both a target tissue, the forestomach, causing a compensatory increase in total glutathione and a decrease in the redox status, and in a non-target, the liver, in which total glutathione was significantly reduced. (This work was supported by NIH contract N01-ES-95437)

704 ACUTE EFFECTS OF DIQUAT ON REDOX STATUS OF HEPATIC COENZYME A AND GLUTATHIONE IN FISCHER-344 RATS

C. V. Smith, K. S. Joppeter-Davis, E. N. Schorr, L. K. Rogers and S. E. Wely. Pediatric, Children's Research Institute, Columbus, OH.

Exposures to oxidant stresses in vivo frequently are characterized by measurements of glutathione disulfide (GSSG), but initiation of irreversible injury has correlated poorly with tissue concentrations of GSSG. Because 70% or more of hepatic coenzyme A is intramitochondrial and CoA and CoASH undergo thiol-disulfide exchange reactions with GSH and GSSG, we measured tissue concentrations of CoA and CoASH, to assess possible compartmental effects in the oxidant stress responses initiated by administration of diquat. Adult male Fischer 344 rats were given 0, 0.05, or 0.1 mmol/kg of diquat, ip. After 2, 4, 6, or 24 h, the animals were anesthetized with pentobarbital, blood drawn, and livers freeze-clamped. Plasma ALT activities were measured as assessments of liver damage, concentrations of CoA and CoASH were measured by HPLC, and hepatic GSH and GSSG concentrations were measured by enzyme recycling methods, as we have described previously. Diquat increased ALT activities, but did not decrease hepatic concentrations of CoA (123 nmol/g of liver in controls), in contrast with the effects of hepatotoxic doses of acetaminophen [Chem Res Toxicol 13:873, 2000]. Diquat caused dose-dependent increases in hepatic CoASH levels that showed significant correlations with plasma ALT activities (P<0.001, r=0.62) in contrast with the effects observed with acetaminophen-induced hepatic injury; however, in no animal did the hepatic CoASH concentrations exceed 10% of the CoASH content. Hepatic GSSG concentrations showed dose-dependent increases and correlated with elevations in plasma ALT activities, although the mean levels in diquat-treated rats were little more than twice the levels observed in saline-treated controls. The present data document the effects of diquat on hepatic CoASH/GCoASH redox status, but do not suggest marked mitochondrial compartmentalization of diquat-induced oxidant stress responses in vivo. Supported by GM44263 from the National Institutes of Health.

705 IMMUNOTOXICITY OF 2-BROMOPROPAINE AND 1,2-DIBROMOPROPANE IN MICE AND RATS.

T. C. Jeong', E. S. Lee', W. Chae', N. H. Kim', W. S. Koh', B. H. Kang' and S. S. Han'. 1College of Pharmacy, Yeungnam University, Kyungkio, South Korea. 2College of Medicine, Taegu Catholic University, Taegu, South Korea. 3Toxicology Research Center, Korea Research Institute of Chemical Technology, Taejon, South Korea. 2-Bromopropane (2BP) is a major component of the mixture of SPG-6AR and Solvent 5200 which is a subspecies produced from diazomethane. In 1995, an occupational exposure to 2BP has been a social issue in Korea. Many female workers in an electronic company were found to have amenorrhea and male workers were diagnosed with oligospermia. In the present studies, immunotoxic effects of 2BP were investigated in male Sprague-Dawley rats and female BALB/c mice. The rats were treated with 2BP at 100, 330 and 1000 mg/kg/day for 28 days. Four days before termination, the rats were immunized with sheep red blood cells (SRBCs). The body and thymus weights were reduced by 2BP at 1000 mg/kg. In addition, the numbers of splenic and thymic cells were decreased by 2BP. In hematology, the number of WBCs, RBCs and platelets was reduced. Among the serum clinical parameters, chloride ion was increased by 2BP. The antibody response to SRBCs was suppressed at 1000 mg/kg. With immunized animals, immunophenotyping of splenic and thymic cells was performed to investigate the changes of the number of macrophages, B- and T-cells in spleen and the number of CD4+ and CD8+ cells in thymus. The numbers of most cell types were decreased in the spleen when animals were treated with 2BP at 1000 mg/kg. Likewise, all cell types of thymus were decreased by 2BP. In a subsequent study, effects of 2BP and 1,2-dibromopropane (1,2-DIBP) on the antibody response were compared in mice. When mice were treated with either one of these chemicals for 7 days, the antibody response was suppressed only by 1,2-DIBP: The present result suggests that 2BP may have an immunotoxic potential in rats, and that 1,2-DIBP contained in the Solvent 5200 may contribute to the immunotoxicity, although 2BP is a major component. (Supported by grant No. 2000-2-21800-001-3 from KOSEF).

706 ETHANOL ALTERS POLY I:C INDUCED NK CELL TRAFFICKING AND ACTIVATION.


Natural killer (NK) cells are critical in resistance to lung tumors establishment in a B16F10 tumor model using female B6C3F1 mice. Polyinosinic-polycytidylic acid (poly I:C, 0.1 mg, ip.) activation of NK cells increases elimination of tumor nodules. This effect is reduced following oral gavage of ethanol (EtOH, 6 g/kg). This study determined if decreased resistance is due to altered trafficking (flow cytometry) and/or NK cell activation (chromium release assay) in the spleen and lungs 4 and 12 h after EtOH and poly I:C administration. Initial studies using anti-CD1- (0.5 mg, ip.) neutralized NK cell depletion revealed 48 h before depletion, 12, 15, and 24 h after B16F10 tumor injection (4x104 i.v.) demonstrate that the majority of tumor cells are cleared by NK cells in 12 h (P<0.001). Flow cytometry revealed that the percentage of NK cells in the lungs at 4 h (62.1%, means±SEM) was equivalent after vehicle, poly I:C and EtOH/poly I:C treatment. At 12 h, however, an increase induced by poly I:C treatment (from 4.1% to 61.9%, p<0.001) was abrogated by EtOH (41%, p<0.01). Corresponding normalized (poly I:C treatment to 100%) NK cell lytic function in the lungs was increased from 26±6 to 100±15% (p<0.001) and 11±1 to 100±7% (p<0.05) following poly I:C treatment at 4 and 12 h, respectively. EtOH reduced this function to 77±21% (ns) at 4 h and 60±8% (p<0.001) at 12 h. Similar results were noted with no difference in percentage of NK cells in the lungs at 4 (5±1) or 12 h (5±1). Lytic function, however, increased from 17±2 to 100±17% (p<0.01) to 4 h and 15±2 to 100±7% (p<0.001) at 12 h. EtOH/poly I:C treatment resulted in decreases to 57±16% (p<0.05) at 4 and 5±3% (p<0.001) at 12 h. These data indicate that EtOH decreases trafficking of NK cells into the lungs 12 h after poly I:C administration, accompanied by reduction in lytic function. Although EtOH did not alter numbers of NK cells in the spleen, it decreased lytic function by half at both 4 and 12 h. This suggests that while EtOH can alter trafficking, it affects NK cells primarily by limiting their activation.

707 IMMUNE STIMULATION FOLLOWING TREATMENT WITH THE HERBAL MEDICINE ECHINACEA PURPUREA IN FEMALE B6C3F1 MICE.


Echinacea Purpurea (EP) is one of the few herbal medicinal plants indigenous to North America. For hundreds of years it has been used by American Indians to treat various ailments. As part of a comprehensive program of the National Toxicology Program, we have been evaluating the safety/hazard characteristics of EP for its ability to modulate the immune system. Female B6C3F1 mice were administered EP daily by oral gavage in a 0.5% methylcellulose vehicle at doses of 30, 100, 300, 600, or 1000 mg/kg for 28 days in range-finding studies and at 300, 600, or 1000 mg/kg in more comprehensive protocol studies. Overall, there were no remarkable effects on the toxicological parameters including, body weight, selected organ weights, or hematological parameters. Similarly, minimal immunological effects were observed. Parameters not affected included, splenocyte differential, mixed leukocyte response (MLR), and natural killer (NK) cell activity. Increases were observed in the spleen IgM antibody-forming cell (AFC) response to sheep erythrocytes at all dose levels; however, the response was not dose dependent and not always statistically significant. Enhanced T cell proliferation was observed following stimulation with anti-CD3 antigen in EP-treated animals. Furthermore, enhanced cytotoxic T lymphocyte (CTL) activity was observed at all effector-to-target ratios in the animals administered 300 mg/kg of EP, while the responses of animals treated with the higher doses of EP were not statistically different than the vehicle controls. Ongoing host resistance studies should be performed if the various components of the immune system stimulated by EP are sufficient to provide protection from various pathogens. (Supported by NIHES Contract ES 53587).

708 COMPARISONS OF JP-8-INDUCED IMMUNE ALTERATIONS FOLLOWING TWO DIFFERENT ROUTES OF EXPOSURE IN FEMALE B6C3F1 MICE.

D. E. Keil, K. Schaefer, J. EuDaly and M. M. Peden-Adams. Medical University of SC, Charleston, SC.

JP-8 jet fuel is used widely in the military and commercial airline environments. Recent reports have implicated jet fuel as an acute immune and respiratory toxicant. Studies via inhalation and dermal exposures have reported similar results; however, oral exposure to jet fuel results in slightly different findings. Previously, no studies have assessed parallel exposures of JP-8 via different routes of administration. In this study female, B6C3F1 mice were exposed to JP-8 jet fuel either orally or dermally for 7 days. The oral dose used was 2000 mg/kg and was administered in a vehicle of olive oil. The dermal dose regime consisted of the application of 50 ul of neat JP-8, an exposure similar to previously published dermal studies. Two control groups were included for the dermal exposure, a group that was administered 50 ul of acetone and another with 50 ul of olive oil. Endpoints assessed included splenic and thymic weight and cellularity, liver weight, urine wet weight, lymphocyte proliferation, natural killer cell activity, T-cell subpopulations, and the antibody plaque forming cell response. Decreases in thymus weight and cellularity were observed following oral exposure but not following dermal exposure. Liver weights
were increased following oral exposure but not following dermal exposure. Uterine wet weight was decreased by both exposure routes but this was not significantly different from the oil control. No effects were observed in NK-cell activity or mitogen-induced lymphocyte proliferation following the exposures. Splenic T-cell subpopulations were altered following dermal exposure. Suppression of the PFC response was observed in the oral JP-8 treatment as compared to its respective olive oil control. Additionally, the PFC cell response was suppressed following dermal exposure as compared to the oil control but not compared to the acetone control. Therefore, the varied findings regarding JP-8 immunotoxicity may be explained in part by the route of administration, as well as the types of controls used in reported studies.

SULFURMID-INDUCED ALTERATIONS IN IMMUNOTOXICOLOGICAL PARAMETERS.

M. M. Peden-Adams, J. G. EuDaly, S. Dabra, L. Hesseman, A. EuDaly and D. E. Keil. Medical University of SC, Charleston, SC.

Fluorinated organic compounds have been manufactured for over 50 years and are currently used throughout industry in semiconductors for computers and other electronic equipment, as stain and water repellents, in floor waxes, popcorn bags, firefighting foam, denture cleaners, carpet spot cleaners, pharmaceuticals, and as pesticides. Manufacture and use of these compounds has steadily increased since the 1970s. Studies show that this class of compounds cause peroxisomal proliferation, hepatomegaly, altered steroidogenesis, and body weight decreases that are associated with a wasting syndrome; however, effects on immune function have not been assessed. This study utilized sulfuramid, a perfluorinated pesticide, as a representative compound from this class of chemicals. Adult female B6C3F1 mice were exposed via gavage to sulfuramid (1, 5, or 10 mg/kg b.w.) for 14 days. Significant decreases in body weight at the high dose (30 mg/kg/d) and increased liver/body weight ratios at 10 and 30 mg/kg/d were noted. Additionally, spleen and thymus weights and cellularity were greatly reduced. Although no differences were observed in natural killer cell activity or mitogen-induced lymphocyte proliferation, suppression was noted in the plaque-forming cell (PFC) response and decreases were observed in thymic T-cell subpopulations. These data indicate that sulfuramid may be a potent immunotoxicant. Further studies are needed to fully characterize the extent of immune parameters affected and the role of the parent compound versus the known metabolite PFOS.

DDT INHIBITS FUNCTIONAL ACTIVATION OF MURINE MACROPHAGES AND DECREASES RESISTANCE TO INFECTION BY MYCOBACTERIUM MICROTI.


DDT is still widely used in several parts of the world to control malaria, typhoid and dengue vectors. DDT has been shown to have immunotoxic effects in mice and to increase susceptibility to intracellular pathogens such as Mycobacterium leprae. However, little is known about the mechanisms underlying this effect. Activated macrophages play an important defensive role against intracellular pathogens, therefore we are trying to evaluate the effects of DDT on the in vitro exposure to technical grade DDT, p,p'-DDT, p,p'-DDE and p,p'-DDD on the functional activation of J774A.1 macrophages and their capacity to limit growth of intracellular pathogens, using Mycobacterium microti as a model. We evaluated cytotoxicity, cell proliferation, functional macrophage activity (NO and O2- production, and mRNA expression of TNF-α, IL-1β and iNOS Synthase), and the ability of treated cells to limit infection with M. microti in IFN-α activated macrophages exposed to 2.5, 5.0 and 10 µg/ml of DDT compounds. Doses of 5 and 10 µg/ml induced direct cytotoxic effects; precluding meaningful analysis of the above parameters, whereas 2.5 µg/ml of all DDT compounds inhibited macrophage activity and reduced their ability to limit the intracellular growth of M. microti without inducing cytotoxicity. Technical grade DDT and p,p'-DDE were the most potent compounds. Therefore, exposure to DDT compounds could represent an important risk for infection development by those intracellular pathogens against which NO and/or O2- production represent the main immune protective mechanism.

ESTROGEN INDUCES THYMIC ATROPHY THROUGH INHIBITION OF CELL CYCLE.

Z. W. Lai, N. C. Fiore and A. E. Silverstone. Microbiology & Immunology, SUNY Upstate Medical University, Syracuse, NY.

Estrogen is a modulator of the immune response. One of the main targets for estrogen in immune system is the thymus. Although recent research suggests that estrogen may inhibit thymocytes at different development steps, the mechanism(s) by which estrogen induces thymic atrophy is still unclear. In the present study, we have determined that inhibition of cell proliferation is one such mechanism. Mice were injected at various times with estradiol (E2) and then B6U was injected 2 hours before mice were sacrificed. Thymocytes were isolated and stained with fluorescein-conjugated antibodies for cell surface and intracellular markers B220 and CD4. Data was acquired using a FACSCalibur flow cytometer and analyzed with FlowJo software for four-color analysis. Our results showed that E2 induced thymic atrophy and reduced the numbers of thymocytes in all CD6+, CD8+, CD4+CD8+ (DP) and CD4−CD8− (DN) subsets. In analysis for cell proliferation, we found that E2 could inhibit DNA synthesis with B6U incorporation in thymocytes and this effect was mainly in CD3−CD4−CD8− DP and CD3−CD4−CD8− TN thymocytes. Furthermore, within the CD3+CD4−CD8− DN subpopulation, E2 mainly inhibited cell proliferation at CD44−CD25+ and CD44−CD25− stages. A time course analysis showed that inhibition induced by E2 appeared as early as 1 day after exposure, whereas the number of total thymocytes was not affected at this time point. The proliferation activity in DP cells returned to normal level, but was still inhibited in DN cells at day 10 after exposure. We also determined the effects of E2 in SCID mice, whose thymuses only contain immature CD4−CD8− DN cells, allowing us to focus our study in the earliest development stages of thymocytes. Our results confirmed that inhibition of cell proliferation was in CD44−CD25+ stage in SCID mice. By using a DNA PI staining for cell size and flow cytometry analysis, our results indicated that E2 could inhibit cell cycle from G0/G1 phase entry into S/G2M phase. Taken together, our results clearly suggest that estrogen induced thymic atrophy is mainly through inhibition of cell proliferation.

DIFFERENTIAL INDUCTION OF APOPTOSIS IN MURINE LYMPHOCYTE SUBPOPULATIONS IN VIVO FOLLOWING CO-EXPOSURE TO LIPOPOLYSACCHARIDE AND VOMITOXIN.

Z. Islam*, L. E. King*, P. J. Fraker* and J. J. Deck*.* Food Science and Human Nutrition, Michigan State University, East Lansing, MI; Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI; Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI; Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.

Lipopolysaccharide (LPS) and vomitoxin (VT, deoxynivalenol) synergistically induce cell death at 12 hr in lymphoid tissue of the mouse, whereas the effects of these toxins alone were negligible. The purpose of this study was to assess the effects of LPS and VT co-exposure on apoptosis within specific T and B lymphocytes subsets in thymus, Peyer's patches, and bone marrow. Flow cytometry revealed that a single dose of LPS (0.1 mg/kg body weight, po) together with VT (12.5 mg/kg body weight, po) resulted in apoptotic death of immature thymocytes (CD4−CD8−), CD4+CD8− and mature thymocytes (CD8+ in thymus with a concurrent loss of cell numbers. R486, a glucocorticoid receptor antagonist, significantly abrogated apoptosis in CD4+CD8−, CD4+CD8+ and CD8+ subsets and also prevented loss in cell numbers. In Peyer's patches and bone marrow, B220+ IgM− IgD− pro-B cells underwent apoptosis at 12 hr after toxin co-treatment. R486 blocked apoptosis in B220+IgM− IgD− subset, however, reduction of cell numbers was still observed at this same time point. Taken together, these data suggest that LPS can interact with VT to induce the glucocorticoid-dependent apoptotic loss of immature thymocytes, cytotoxic T lymphocytes and pro-B cells in mouse lymphoid organs.

IMMUNOMODULATORY EFFECTS ASSOCIATED WITH ANTI TUMOR NECROSIS FACTOR (TNF) ANTIBODY TREATMENT IN THE FEMALE B6C3F1 MOUSE.


Tumor Necrosis Factor Alpha (TNF-α) is responsible for stimulating numerous biologic functions including the inflammatory response, as well as enhancing the production of other cytokines involved in both immune and non-immune processes. Inhibition of TNF-α has been shown to have therapeutic benefits for the treatment of rheumatoid arthritis by reducing the associated inflammation and tissue degradation. Recently clinical data from inhibitors of TNF-activity (e.g., Enbrel and Remicade) suggest that these drugs may produce certain undesired immunomodulatory effects (e.g., increased incidence of infections). To understand the potential effects of TNF-inhibition on innate and acquired immunity, a 4-week immunotoxicology study was conducted in female B6C3F1 mice administering anti-murine TNF-αantibody once a week at approximately 250 µg/dose by intraperitoneal (IP) injection. In addition to standard toxicity endpoints (including body weights, clinical observations, and spleen, thymus, liver, and kidney weights), spleen IgM and IgG body response to a T-dependent antigen, sheep erythrocytes, natural killer (NK) cell activity, and splenic and peripheral blood lymphocyte subsets were evaluated. Cyclophosphamide and Anti-Asialo GM1 (Rabbit) antibody were used as positive controls. No treatment-related effects on body or organ weights were observed.
Anti-TNF Ab treatment resulted in an approximate 35% decrease in IgM AFC response/107 splenocytes and an approximate 30% decrease in the absolute numbers of peripheral blood suppressor/cytotoxic T-cells (CD3+CD8+). No treatment-related effects in peripheral blood or splenic total B and T cells, splenic macrophages, or splenic T-cell subsets were noted. Although not statistically significant, NK cell activity was increased when compared with the vehicle control. These results suggest that inhibition of TNF activity may affect the acquired immune response and therefore may contribute to the immunomodulatory effects seen with the inhibitors of TNF activity.

714 DIESEL EXHAUST PARTICLES INCREASE LUNG INJURY AND INFLAMMATION DURING EARLY SENSITIZATION IN BROWN NORWAY RATS.

P. Singh1, M. J. Daniels1, D. Winnest1, J. Richar3, L. Bisham1, C. A. Dick1, M. Madisen1, K. B. Adler2 and M. L. Gilmour3, 1CVM, NCSU, Raleigh, NC, 2HH, Chapel Hill, NC.

Diesel exhaust particles (DEP) possess demonstrated adequate activity in both human and experimental respiratory allergy, yet the mechanisms of adjuvancy remain poorly described. We have previously reported that intratracheal (IT) instillation of DEP to rats prior to allergen sensitization enhances pulmonary allergic responses to house dust mite (HDM) in Brown Norway (BN) rats. The purpose of the present study was to track the adjuvant effects of DEP during early sensitization to HDM. BN rats were instilled IT on day 0 with either 100 or 500 μg DEP (SRM 2975) or saline only and immunized IT 24 hr later with 10 μg HDM or saline ( sham-immunized) on days 1 and 3. Lung injury and inflammatory status were assessed after sacrifice on day 7. Tissue was separated into cell fractions and analyzed for cytokines. DEP (500 μg) significantly increased total protein, lactate dehydrogenase (LDH) and total inflammatory cell numbers in bronchoalveolar lavage (BAL) fluid in both HDM and saline-treated rats. Specifically, DEP (500 μg) induced a sharp increase in the total number of neutrophils in the BAL fluid, peaking at 6 hr and remaining significantly elevated at 24 hr after the final immunization. DEP (500 μg) also increased total numbers of exosomes in the BAL fluid at 24 hr. In order to account for DEP-enhanced inflammatory cell influx during early sensitization, we quantitated chemokine levels in BAL fluid and lung homogenate at these time points. The kinetics of chemokine production in response to DEP exposure indicated an early increase in MIP-2 and RANTES at 6 hr, followed by a decrease at 24 hr after the final immunization in both HDM- and sham-immunized rats. These results show that DEP causes an early increase in lung injury and inflammatory cell influx and a coincident shift in local chemokine production, which may contribute to activation of specific immune responses. (Supported by NCSU/UEA Training Agreement CTR 0512010 and NIH grant # ES11245-01) (This abstract does not reflect EPA policy.)

715 TIME DEPENDENT INCREASE IN ALVEOLAR MACROPHAGE PHAGOCYTOSIS FOLLOWING INCUBATION WITH CARBON BASED PARTICLES.

C. A. Dick1, M. Daniels1, S. Becker1, P. Singh1, M. J. Salgrade1 and M. L. Gilmour3, 1Center For Environmental Medicine and Lung Biology, UNC, Chapel Hill, NC, 2Experimental Toxicology, HH, Chapel Hill, NC, 3Clinical Toxicology, HH, Chapel Hill, NC.

Epidemiological studies have shown that a rise in PM(10) is associated with exacerbations of respiratory disease, increases in respiratory infection and increased morbidity. The mechanism of these effects are not well understood, but the ability of the lung to protect and remove inhaled particles will determine the outcome in terms of adverse health effects. We investigated whether exposure to carbon based particles could alter subsequent phagocytic activity. AM, isolated from rats, were exposed in vitro for 4 or 24 hours to varying doses of fine carbon black (CB), ultratine carbon black (UCFB), carbon black coated with 5% copper sulfate (CBCu), carbon black coated with a mixture of 1% iron, copper, vanadium and nickel sulphates (CBM1S) and diesel exhaust particles (Department) (10, 15, 25 and 50ug/100, 000 cells). Secondary phagocytosis was assessed over a 3 hour period by monitoring ingestion of FITC-labeled E. coli. After 2 hour incubation, all of the particle types increased secondary AM phagocytosis regardless of dose. This increase was sustained following 4 and 24 hours of particle incubation with the CBM1S particles, but not with the other particle types which decreased to control levels. Biochemical analysis of supernatants revealed no change in LDH and marginal increase in total protein in all particle controls. Lysosome levels were elevated after the 4 hour treatment period with all particles, compared to control levels. TNF alpha protein levels were also elevated 15-20% after 24hr incubation regardless of concentration or type of particle used. The data show that short term incubation with carbon based particles increases secondary AM phagocytosis with subsequent release of bacterial endotoxins and pro-inflammatory cytokines. The results suggest that host defenses may be activated in response to environmental insult. This abstract does not reflect EPA policy.
caused increases in spleen weight and spleenocyte population. The spleenocytes from 
aniline-treated rats showed greater mitogenic responses to concanavalin A and 
pokeweed mitogen, as determined by MTT assay. interleukin-1 (IL-1), tumor 
necrosis factor-alpha (TNF-alpha) and transforming growth factor-beta 1 (TGF-
beta 1) in the culture supernatants of spleenocytes (cultured for 24 h) from 
control and aniline-treated rats were quantitated using specific ELISA kits. All three 
cytokines, i.e., IL-1 TNF-alpha and TGF-beta 1 showed increased secretion in the 
culture supernatants from aniline-treated rats as compared to the controls. Greater 
induction of IL-1, TNF-alpha and TGF-beta 1 in the spleenocytes from aniline-
treated rats, along with morphological evidence of fibrosis, suggest a role for these 
cytokines in the aniline-induced splenic fibrosis.

719 PERINATAL EXPOSURE TO ATRAZINE ALTERS JUVENILE IMMUNE FUNCTION IN SPRAGUE-
DAWLEY RATS.
A. A. Rooney and R. W. Ludtke. Anatomy, Physiology, Science, 
and Radiology, NCSU/SEPA, Research Triangle Park, NC and NIEHS, 
USEPA, Research Triangle Park, NC.

High doses (50-300mg/kg) of the chloro-triazine herbicide atrazine (ATR) alter 
edocrine function (e.g., lactating hormone and triiodothyronine secretion) in juve-
nile or adult rats; however, ATR appears to have no effect on adult immune func-
tion. Exposure of nursing dams to ATR produces persistent changes in the 
immunomodulatory hormone prolactin in their offspring, reflecting the familiar 
trend of greater neonatal susceptibility. Furthermore, the immunoregulatory prop-
cesses of prolactin and thyroid homones suggest that ATR may have perinatal 
immunomodulatory potential: either directly or through transitory hypoprolactinemia 
or hypothroidism. Therefore, to evaluate ATR as a developmental immunotoxican-
ti, pregnant Sprague-Dawley rats were subjected to 4 separate treatment regimes 
from gestational day 10 through post-natal day 21 (PND 21): 1) ATR (35mg/kg/day) 
in methyliccelulose via gavage, 2) bromocriptine (0.2mg/kg/2x/day) to induce 
hypothyroidism, 3) propylvithoracin (2mg/kg/day) to induce hypothy-
roidism, or 4) methyliccelulose v/gavage for control dams. Offspring were 
them then tested after PND 30 for: 1) natural killer (NK) cell function, 2) delayed-type 
 hypersensitivity (DTH) response to bovine-serum albumin (BSA), and 3) phagecyto-
sis of lysis beads by peritoneal macrophages. Exposure of pregnant dams to ATR 
decreased NK cell function and DTH responses in male offspring but increased 
NK function in female offspring. These results demonstrate that perinatal 
exposure to ATR produces gender specific changes in immune function that persist 
into adulthood. Although transitory hypothroidism increased phagocytic response 
in offspring, neither hypothyroidism or hypoprolactinemia altered NK or DTH 
responses, suggesting that immune changes associated with ATR are not mediated 
through suppression of these systems. (This abstract does not reflect EPA policy 
and was supported in part by the NCSU/SEPA Cooperative Training Program in 
Environmental Sciences Research, Training Agreement C1820S01010 with North 
Carolina State University).

720 ESSENTIAL ROLE OF DOUBLE-STRAINED RNA-
ACTIVATED PROTEIN KINASE PKR IN VOMITOXIN-
INDUCED MAPK PHOSPHORYLATION, TNF-ALPHA 
PRODUCTION AND APOPTOSIS IN HUMAN U-937 
CELLS.
H. R. Zhou and J. J. Pestka. Food Science and Human Nutrition, 
Michigan State University, East Lansing, MI and Institute of Environmental 
Toxicology, Michigan State University, East Lansing, MI.

Vomitoxin (VT, deoxynivalenol) and other trichothecene mycotoxins inhibit trans-
lation and trigger a ribosome stress response that results in phosphorylation of mi-
trogen-activated protein kinases (MAPKs) as well as induction of TNF-alpha ex-
pression and apoptosis in cloned murine macrophages. Previous studies with 
the chemical inhibitors 2-aminopurine and adenosine have suggested that activation of 
double-stranded RNA-activated protein kinase (PKR) are involved in these events. 
The purpose of this study was to employ a stable transformant of human promono-
cytic U-937 cells containing an actin promoter expression vector (CUB cells) to 
evaluate the roles of PKR in mediating VT-induced MAPK activation, translational 
arrest, TNF-alpha expression, and apoptosis. Significant reduction and delay of 
VT-induced phosphorylation of MAPKs was observed to occur in a dose-dependent 
fashion in CUB cells as compared to control cells transfected with vector only 
(CUC2 cells). Specifically, JNK 1 and JNK 2 phosphorylation was inhibited com-
pletely in CUB cells. ERK 2 (p42) but not ERK 1 phosphorylation was reduced 
whereas p38 was only partially suppressed. The LD50s for leucine incorporation 
in cells treated with VT or the transitional inhibitors anisomycin (ANI) and emetine 
(EM) were increased by 25 to 50% in CUB as compared to CUC2. Similarly, 
stimulation indices for VT-induced TNF-alpha expression were markedly decreased in 
CUB as compared to CUC. Both capric activation and DNA fragmentation following VT,
AN and EM exposure were markedly suppressed in CUB as compared to CUC2. 
The results suggest the PKR may be essential for VT-induction of MAPK phos-
phorylation and for resultant elevations in cytokine production and apoptosis in leu-
ocytes (Supported by NIEHS grants ES-05358 and ES-05535).

721 EVIDENCE FOR THE INVOLVEMENT OF THE CB2 
RECEPTOR IN THE MODULATION OF INTRACELLULAR 
CALCIUM ([Ca2+]i) BY Δ°-TETRAHYDROCARBINOIN 
(Δ°-THC) IN HUMAN T CELLS.
G. K. Rao, E. L. Sawyer and N. E. Kaminski. Pharmacology & Toxicology, 
Michigan State University, East Lansing, MI and NIEHS, NIH, 
Bethesda, MD. (Supported by NIEHS grants ES-05358 and ES-055358).

Cannabinoids are a class of over 60 structurally-related plant-derived compounds 
present in the marijuana plant, Cannabis sativa. Cannabinoids are potent inhibitors 
of IL-2 production in T cells. In the present studies, HBF-ALL and Jurkat E6-1 
human T cell lines were employed to elucidate the mechanism of IL-2 inhibition by 
Δ°-THC, the primary psychoactive cannabinoid in marijuana. Previous work 
from this laboratory has demonstrated by Northern analysis that both cell lines lack 
transcripts for the central cannabinoid receptor, CB1; and that the transcript for 
peripheral cannabinoid receptor, CB2, was normal sized (2.4kb) in HBF-ALL cells, 
but aberrant in Jurkat E6-1 cells (2.3, 4.5 and 5.7kb). The objective of the present 
study was to examine the effect of Δ°-THC on [Ca2+]i, and inhibition of phospholip 
ester (PMA) and ionomycin (Io) induced IL-2 production in HBF-ALL and Jurkat 
E6-1 cells. Δ°-THC (10μM) induced a rapid and transient rise (500-700nM) in 
[Ca2+]i, in HBF-ALL cells as compared to a slow and modest rise (80-100nM) in 
Jurkat E6-1 cells. This rise in [Ca2+]i was maintained in presence of the CB2 recep-
tor antagonist, SR144528 (1μM), in HBF-ALL cells, but not in Jurkat E6-1 cells. 
In addition, Δ°-THC inhibited IL-2 secretion in HBF-ALL cells in a concentration 
dependent manner, but not in Jurkat E6-1 cells. These data suggest that Δ°-THC 
acts to disrupt [Ca2+]i regulation in a CB2 receptor-dependent manner which may 
contribute to the inhibition of IL-2 secretion. (Supported in part by NIH grant 
DA07390).

722 PARADOXICAL MODULATION OF T CELL GROWTH 
BY THE ENDGENOUS CANNABINOID, 
2-ARACHIDONYL GLYCEROL, CAUSES AN INHIBITION 
OF THE T CELL GROWTH FACTOR, IL-2, AND A 
CONCURRENT ENHANCEMENT OF T CELL 
PROLIFERATION.
C. E. Rochwell and N. E. Kaminski. Pharmacology & Toxicology, Michigan State 
University, East Lansing, MI.

The putative endogenous cannabinoid, 2-arachidonyl glycerol (2-AG), is an arachi-
donic acid derivative that has been shown through radioligand binding studies, 
to bind with affinity to the cannabinoid receptors, CB1 and CB2. 2-AG mimics some 
of the immunomodulatory effects of the plant-derived cannabinoids, Δ°-THC and 
cannabinoids, including inhibition of cytokine production. The first objective of 
the present study was to determine the role of the CB1 and CB2 receptors in 2-AG-
mediated inhibition of IL-2. In activated murine splenic and thymic T cells, 2-AG 
carries a marked and concentration-responsive inhibition of IL-2 that is more po-
tent than anandamide, another putative endogenous cannabinoid. The CB1 and 
CB2 antagonists, SR141716A and SR144528 respectively, did not antagonize the 
inhibition of IL-2 mediated by 2-AG (unpublished). The second objective of the 
present studies was to determine the effect of 2-AG upon T cell proliferation. 
Interestingly, 2-AG causes a concurrent concentration-responsive enhancement of 
T cell proliferation at the same concentrations at which it inhibits the production 
of IL-2. 2-AG caused a significant enhancement of T cell proliferation at 1μM 
and an approximate 50% increase in proliferation at 10μM. Neither anandamide nor 
arachidonic acid caused an enhancement of T cell proliferation. These data suggest 
that 2-AG enhances T cell proliferation while paradoxically and concurrently 
hibiting the production of the T cell growth factor, IL-2, in activated murine 
splenic and thymic T cells. These data also suggest that the 2-AG-mediated 
enhancement of T cell proliferation is unique to 2-AG and not common to arachi-
donic acid and its derivatives. (Supported in part by NIH Grant DA 12740).

723 CONCENTRATION-DEPENDENT BIFUNCTIONAL 
effects of TGF-β, on Immunoglobulin Production: Role for Smads2/3 in IgA Production in vitro.
S. C. McKenna, J. J. Liston and N. E. Kaminski. Lab of Cell Regulation 
and Cytokines, NCI, NIH, Bethesda, MD and Pharmacology and Toxicology, 
Michigan State University, East Lansing, MI.

TGF-β is a critical regulator of humoral and cell-mediated immune homeostasis. 
The molecular mechanisms responsible for the regulation of immune cell function 
extended by TGF-β, are not well defined. We demonstrate bifunctional augmen-
tion and inhibition of in vitro T cell-dependent anti-SRBC and T cell-independent DNP-Ficoll-induced AFC responses by fentanyl and picomolar concentrations of TGF-β, respectively. To investigate the molecular mechanisms responsible for the inhibitory effects of TGF-β on T cell-dependent and -independent AFC responses, spleenocytes were obtained from mice homografts for a null mutation in the gene encoding the TGF-β receptor-activated Smad3 (Smad3−/−) and sensitized in vitro to either anti-SRBC or LPS. Inhibition by TGF-β was markedly reduced in the Smad3−/− B cells compared with age-matched wild type littermates (Smad3+/−). Moreover, LPS-sensitized Smad3−/− B cells were less sensitive to TGF-β-induced inhibition of IgM secretion as determined by ELISA. A role for Smad3 in Ig production in vivo was also investigated. TGF-β did not induce IgA production in LPS-sensitized Smad3−/− B cells under conditions that yielded significantly increased IgA production Smad3+/− B cells. In conclusion, our results demonstrate a bifunctional, concentration-dependent effect of TGF-β on Ig production. These results are also provide evidence for a role by Smad3 in IgA production by TGF-β, in vitro.

724  ENHANCEMENT OF TNF-α mRNA STABILITY BY DEOXYVINALENOL THROUGH ACTIVATION OF PKB KINASE.

Y. Chung,1,2 and J. L. Presta.1,3 Food Science and Human Nutrition, Michigan State University, East Lansing, MI; Institute for Environmental Toxicology, Michigan State University, East Lansing, MI; Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.

Deoxyvinalenol (vinorelbin, VT), a common fungal metabolite in grapes, up-regulates proinflammatory cytokine gene expression. This up-regulation is controlled by both transcriptional and post-transcriptional mechanisms. We have recently shown that VT up-regulates TNF-α gene expression post-translationally via enhanced mRNA stability. Since stabilization of cytokine mRNA is often related to activation of mitogen activated protein kinases (MAPKs), we investigated the role of these kinases in enhanced TNF-α mRNA stability in RAW 264.7 macrophages. p38 kinase, ERK1, and ERK2 were found to be activated by VT. The effects of VT on increased mRNA stability in the presence of MAPK inhibitors were studied in lipopolysaccharide (LPS)-stimulated cells. After 4 hr stimulation by LPS, a transcription inhibitor, 5, 6-dichloro-1-beta-D-ribofuranosyl benzimidazole (DRB), with or without MAPK inhibitor (SB203580 or PD98059) was added to culture and VT-mediated changes in TNF-α mRNA stability were measured using a ribonuclease protection assay (RPA). Increased TNF-α mRNA stability by VT was abrogated in the presence of p38 kinase inhibitor (SB203580). Treatment of ERK inhibitor (PD98059) did not affect stabilization of TNF-α mRNA by VT. These results suggest that VT up-regulates TNF-α gene expression by increasing mRNA stability via activation of p38 kinase.

725  EXTRACELLULAR METALLOTHIONEIN ALTERS THE T RESPONSE TO COGNATE ANTIGEN PRESENTED BY ANTIGEN PRESENTING CELL.

K. C. Cowherth and M. A. Lysaght. Department of Molecular and Cell Biology, University of Connecticut, Storrs, Mansfield, CT.

Metallothionein (MT) is a stress-induced protein that can modulate several key immune responses. In previous work, MT has been demonstrated to decrease in vivo and in vitro measures of humoral and cell-mediated immunity. MT has also been shown to bind to leukocyte plasma membranes both in vivo and in vitro. These observations suggest that MT may alter cell function through interaction with surface proteins preventing effective cell-cell interaction. We have used antigen presenting and cell proliferation assays to address this possibility. Our results show that MT can significantly interfere with in vitro antigen recognition by T cells co-cultured with antigen presenting cells (APCs). In these experiments, MT decreased OVA-stimulated DO-11.10 T cell cytokine secretion by 20-30% when compared to vehicle control. This MT-mediated suppression of T cell responses also occurred in T-rich cultures. When co-cultured with added cells, indicating that the induction of intracellular MT is not required for the mechanism of suppression. Furthermore, using an array of H-2a restricted T cell lines that are each specific for different OVA epitopes, we observed MT-mediated suppression of IL-2 and IL-4 cytokine secretion in a majority of clonal T cell responses to antigen presented by APCs. Intriguingly, there was one OVA-specific T cell clone response that was enhanced by the presence of MT. DO-11.10 T cell responses were also suppressed in the presence of MT when stimulated by fixed APCs, suggesting that MT need not be present during antigen uptake and processing to suppress subsequent T cell responses. T cell proliferation that is experimentally induced with anti-CD3 antibodies is also suppressed by the presence of MT, suggesting that MT may interfere with T cell responses through the T cell receptor/MHC immune synapse. These observations further extend the idea that metallothionein synthesis as a consequence of cell stress may play an important role in the onset of stress-induced immune dysfunction.

726  SODIUM METHYLDITHIOCARBAMATE SUPPRESSES INTERLEUKIN-2, INTERLEUKIN-4, AND INTERFERON-γ PRODUCTION AFTER STIMULATION IN B6C3F1 MICE.

L. P. Myers and S. B. Pfeffer. Department of Cellular Biology and Anatomy, Louisiana State University Health Sciences Center, Shreveport, LA.

Sodium methyldithiocarbamate (SMDC) is a commonly used fungicide, nematocide, and herbicide in agricultural soils, which is immunotoxic in mice. Since other dithiocarbamates (pyrolylindithiocarbamate, e.g.) have been implicated in cytokine overproduction in cancer patients, we investigated the effect of SMDC on cytokine production when pre-treated with SMDC (300 mg/kg) and then stimulated with anti-CD3 (3 μg/mouse). B6C3F1 mice showed marked suppression of serum cytokine production at 90 minutes post-stimulation. SMDC suppressed Interleukin-2 (IL-2) (1323.2 pg/mL) compared to anti-CD3 alone (4635.2 pg/mL). Similar SMDC suppressed productions were seen with IL-4 (643.2 compared to 1091 pg/mL), and Interferon-γ (INF-γ) (1398.0 compared to 5361.8 pg/mL). Expression of IL-2, IL-4, IL-13, and INF-γ mRNA was significantly suppressed by SMDC at 45 minutes, but the cytokines were not significantly different at 90 minutes post-stimulation when evaluated by RNAase protection assays. Since SMDC increases serum corticosterone by inducing a stress response, aminoglutethimide was administered to evaluate the role of glucocorticoids in cytokine suppression. Although the overall levels of cytokines were increased (possibly due to the lack of corticosterone to regulate the cytokine expression), a similar pattern of cytokine suppression was noted when SMDC was administered in the absence of corticosterone. Therefore, it is unlikely that corticosterone plays a role in SMDC-induced cytokine suppression. Since other dithiocarbamates suppress cytokines by blocking NF-kB signaling, we evaluated the effect of a single dose of SMDC (300 mg/kg) over a time course. SMDC depleted basal levels of NF-kB in the nucleus of splenic T-cells by 50% at 60 minutes post-administration. NF-kB levels then increased to normal by 80-120 minutes after administration of SMDC. Therefore, it is possible that SMDC suppresses cytokine expression by blocking NF-kB signaling in the cell. This work was supported by NIH grant #5R01CA15818.

727  LOW CONCENTRATIONS OF INORGANIC MERCURY INHIBIT RAS ACTIVATION DURING T CELL RECEPTOR MEDIATED SIGNAL TRANSDUCTION.

A. J. Rosenspire, A. Felcak, C. C. Chen, M. J. McCabe Jr and R. R. Maring 1st. Biological Sciences, Wayne State University, Detroit, MI and Environmental Medicine, University of Rochester, Rochester, NY.

Mercury is widespread in the environment, and consequently there are large populations who are currently exposed to low levels of mercury as a result of ubiquitous environmental factors. Whether these environmental levels of mercury are harmful is a matter of current debate, with epidemiological and animal studies suggesting detrimental effects on the immune and nervous systems. However specific cellular effects of low concentrations of mercury have been difficult to characterize. We now demonstrate that sub-toxic concentrations of HgCl₂ can potently (maximal at 1 μM) increase RasGTP levels inJurkat, a human T cell line. Remarkably, this activation of Ras occurs without a concomitant increase in MAP kinase activation, suggesting that mercury may directly produce a non-parametric effect on the Ras pathway. In addition to its direct effect on Ras, concentrations of HgCl₂, as low as 0.6 μM inhibited the ability of the T Cell Receptor to activate Ras and MAP kinase. The inhibitory effect of mercury is selective, as activation of MAP kinase by phorbol diesters remain intact. Since the Ras/MAP kinase pathway is both highly conserved, and central to signal transduction processes mediated by a myriad of diverse membrane receptor systems in a variety of cell types, these results suggest a mechanism for adverse health effects resulting from exposure to low levels of mercury. They also support a model for regulation of the Ras/MAP kinase pathway, whereby partial but unproductive activation of Ras can diminish signaling from cell surface receptors.

728  MECHANISMS OF PPARY-MEDIATED PRE-B CELL APOPTOSIS.


The role of PPARγ in adipocyte differentiation and function is well recognized. In addition, PPARγ is expressed strongly in the immune system and also influences immune cell function. As shown by propidium iodide staining, DNA ladder formation, and PARP cleavage, PPAR γ agonists (15-deoxy-d12,14-prostaglandin J2, ciglitazone, clobiglitazone and rosiglitazone) rapidly (6 hours) induced apoptosis in pro/pre-b cells (BU-11), immature B cells (WEHI-231) and plasma cells (HS Sultan, U266, IM9). We hypothesized that activation of PPARγ by its dimerization partner retinoid X receptor (RXR) induces B cell apoptosis. Untreated B cells express PPARγ only in the cytoplasm. Following treatment with ciglitazone, the receptor translocates to the nucleus, binds a consensual PPARγ binding, nucleotide
sequence derived from the acetyl CoA oxidase promoter, and initiates transcription, as indicated by western blot, EMISA, and increased PPARδ-dependent reporter activity. The role of PPARγ in apoptosis is supported further by the fact that co-treatment with 9-cis retinoic acid (a specific agonist for RXR) synergistically increased the incidence of apoptotic B cells. Given previous studies indicating that PPARγ-induced pre-B cell apoptosis is triggered by NF-kB down-regulation, we also hypothesized that alteration of NF-kB-DNA binding is involved in PPARγ agonist-induced apoptosis. Ciglitazone caused a 3.6-fold increase in NF-kB binding to the kB site in the upstream regulatory element of c-myc. NF-kB-DNA binding increased as early as 1 hour post-treatment and was maximal 6-7 hours post-treatment: the p50, p65 and c-Rel subunits of NF-kB all contributed to this increase. In parallel with the apoptosis dose-response curves, co-treatment with 9-cis retinoic acid and ciglitazon decreased the ciglitazone dose required to activate NF-kB, suggesting that PPARγ-RXR heterodimer activation results in NF-kB activation. In summary, activation of PPARγ appears to initiate apoptotic signaling in B cells of multiple maturation stages, potentially through the activation of NF-kB.

**729**

**CUMULATIVE IRRITATION (21 DAY) STUDY OF POLYVINYLIDENE FLUORIDE COATINGS ON HUMAN SUBJECTS.**


Introduction: Our laboratory has developed a unique formulation, Epikote™ coatings, for direct application to skin. This blend of polyvinylidene fluoride (PVDF) and acrylic polymers, is safe and remains intact on human skin for extended periods of time, making it beneficial in the prevention of irritation due to incontinence. Methods: PVDF coatings were applied in a randomized, cross-over design to the backs of 26 human subjects over 21 day period and scored for irritation. Subjects were 21 to 75 years of age, had no clinically significant history of skin, kidney, liver, lung, or diabetes and no evidence of any skin lesions. Subjects served as their own controls. Each subject had 10 sites of 4x4 cm2 areas on their mid-back, beneath the angles of the scapulae, demarcated with an indelible skin marker. Subjects reported for treatment and evaluation daily throughout the week. No treatment or evaluation took place over weekends. During daily treatment, coatings were removed, skin was scored for irritation, and the coatings were reapplied. Daily irritation scoring was made 24 hours after each application of the PVDF coating test material (except weekends). After scoring, the treatment area was cleaned with soap and water and air dried, and coatings were reapplied to the same sites. Visual scoring was made for erythema/eschar (0 - no erythema to 4 - severe erythema/slight eschar formation) and edema (0 - no edema to 4 - severe edema/raised more than 1 mm). In addition, non-invasive testing at each test site was performed using three instruments: a colorimeter which measures the color of the skin and erythema; an evaporimeter which measures the moisture content of the skin; and a laser-Doppler, which measures the microcirculatory blood flow. Results: No evidence of any erythema or edema was found on any subject at any time. There was never a score greater than 0 on any site during the 21 day study. Finally, none of the instruments showed any adverse effects. Conclusion: PVDF coatings are non-irritating to humans.

**730**

**IMMUNOMODULATION FOLLOWING ORAL ADMINISTRATION OF THE ANTIFUNGAL AGENT SCh 65592.**


SCh 65592 is a broad-spectrumazole antifungal drug in development for treatment of mucosal and systemic fungal infections. Immunocompetence of CD-1 mice following daily dietary dosing of 10, 30 and 90 mg/kg SCh 65592 for 1 and 3 months was assessed. A 1-month recovery period following 3 months of dosing was also included. Immunotoxicity assessment included lymphoid organ weights and cellularity, hematologic, leukocyte phenotype analysis of blood and spleen, induction of antibody-forming cells (AFC), and natural killer (NK) cell activity. Animals dosed with SCh 65592 had an increased incidence of a common background ear lesion in CD-1 mice, but no changes in body weight or food consumption. Dose-related increases in spleen, liver and adrenal weights and lymphopenia occurred; thymus weight and cellularity were unaffected. There were mild to moderate increases in leukocytes due to increases in monocytes, neutrophils and/or lymphocytes at 30 and 90 mg/kg after 1 and 3 months of dosing; these increases were still apparent 1 month postdose at 90 mg/kg. There was a dose-related trend toward increased NK cell number and monocyte number and percentage; these changes resolved following recovery. Splenic lymphocytes were also increased. SCh 65592 had no effect on AFC production in males; females were unaffected at 1 month, but at 3 months they exhibited a statistically significant increase in AFC at 10 mg/kg, and a statistically significant decrease in AFC at 90 mg/kg. Changes in CD-1 mice following oral administration of SCh 65592, a broad-spectrum azole antifungal drug, were minimal, and resolved following recovery. NK activity was increased in both sexes at both 30 and 90 mg/kg following a 1-month administration, and in males only following 3 months of dosing. Following recovery, NK activity returned to control levels in all groups. These data suggest that administration of SCh 65592 is associated with modest immunomodulation in CD-1 mice. The resolution of most changes following cessation of dosing suggests that SCh 65592 has no permanent effect on the functional integrity of the immune system.

**731**

**ASSESSMENT OF IMMUNOMODULATORY POTENTIAL OF FTY720 IN A MURINE CONTACT ALLERGY MODEL.**


FTY720 is a chemically modified fungal product, which is in development for the use in organ transplantation to prevent allograft rejection. Contact allergy (CA) was induced by a 3 day topical treatment with dinitrochlorobenzene (DNB) on the shaved back of BALB/c mice. 12 days later, elicitation of CA was provoked by a 3 day topical application of DNCB on the dorsum of both ears. FTY720 was administered orally during the elicitation phase in sensitized mice and the concurrent non-sensitized control animals. Necropsy was performed one day after the last treatment. In a preceding experiment FTY720 caused a marked reduction of peripheral lymphocyte counts and a marked decrease of ear-draining lymph node (LN) weights at 0.1 and 1 mg/kg, when applied for 3 days together with topical application of DNB. Oral application of FTY720 1 mg/kg FTY720 during elicitation of CA led to a decrease in the DNBC-caused hyperplasia of ear-draining LN and a reduction in the allergy-relevant increase in ear weight. However, evaluation of IL-2 receptor expression on the surface of CD4+ T cells and the generation of CD4+ T cell blasts by flow cytometry of cells from the ear-draining LN did not reveal significant differences compared to the control group. In contrast, the immunosuppressant FK506 caused a marked reduction in this study not only of the LN hyperplasia and allergy-relevant ear swelling, but also of IL-2R expression and CD4+ T cell blastocytosis. In addition, the cytokines IL-2, IL-4 and IFN gamma were determined in supernatants of restimulated LN cells derived from FTY720 and DNCB-treated mice. No substantial changes caused by FTY720 in the DNBC-induced pattern of these cytokines were observed. The presented findings correspond with the mechanism of action of FTY720, which prevents immune-mediated rejection of allografts by distracting lymphocytes from the periphery rather then by suppressing their activation. This study shows that a contact allergy-based murine model can be applied to investigate the influence of test articles on the immune systems function.

**732**

**GENISTEIN MODULATES NK CELL ACTIVITY, ANTICD3-MEDIATED T CELL PROLIFERATION AND PHENOTYPIC MARKER EXPRESSION BY THYMOCYTES AND SPLENOCYTES IN F3 AND F8, GENERATIONS OF C57BL/6 MICE.**


Although the phytoestrogen genistein (GEN) has been hypothesized to have beneficial health effects in adults, concern exists about the long-term adverse effects of this compound. The objective of the present study was to evaluate the effect of GEN on the immune system in adult and developing mice. Pregnant C57BL/6 mice were exposed to GEN at low (L: 25 ppm), middle (M: 250 ppm) and high (H: 1250 ppm) concentrations in feed starting on day 14 of gestation. The offspring were exposed to GEN gestationally and lactationally, and to GEN-containing feed after weaning on postnatal day 21. Immunological evaluation was performed on postnatal day 42. In dams, exposure to GEN increased terminal body weight (M and H), splenic T-cell and NK cell numbers (M), and the activity of NK cells (M). In F1 males, GEN increased terminal body weight and spleen weights (L and M), the number of CD4+CD8+ and CD4+CD8- thymocytes (L), and the number of spleen T-cell subsets and NK cells (L and M). Moreover, splenic NK cell activity and anti-CD3-mediated splenocyte proliferation were increased in all dose groups. In F1 females, the percentages of CD4+CD8+ and CD4+CD8- thymocytes (L and M), and the splenic CD4+CD8- and CD4+CD8+ T cells (L and M) were increased. In contrast, the percentage and number of CD4+CD8+ thymocytes were decreased in the middle dose group. Exposure to GEN decreased the percentages of splenic NK cells in all dose groups, while decreased the activity of splenic NK cells in the low dose group. Overall, these results demonstrate that GEN can modulate the immune system with greater effects occurring in developing mice. The sexual dimorphic effects of GEN on the immune responses of F3 male and female mice suggest that GEN may function as an endocrine disruptor. (Supported in part by the Jeffress Memorial Trust and NIEHS Contract ES 55387).
733 STRAIN-DEPENDENT PRIMARY AND SECONDARY DICLOFENAC-INDUCED POPITLEAL LYMPH NODE REACTIONS.

B. W. Curtin, F. Bouazahra, P. Kong and J. E. Craft. Yale University School of Medicine, New Haven, CT.

Diclofenac (DF) is associated with immune-mediated drug hypersensitivity reactions (IDHRs). Although the mechanisms causing DF-induced IDHRs remain to be defined, it is thought that IDHRs are controlled by genetic factors. The aim of this study was to examine the immunomodulating potential of DF in two genetically diverse and MHC-mismatched mouse strains (BALB/c, H2d and B6, H2b). This study used a popliteal lymph node assay (PLNA) that was conducted in naive and orally pretreated mice (3 weeks after a single dose of DF). In naive mice, DF induced a PLN reaction in both strains that resulted in the accumulation of activated T cells in the PLN. Despite these similarities, strain-dependent effects were observed. For example, there was an increase in the % of CD3+CD4+, and CD3+CD8+ lymphocytes in B6 mice, whereas no increase was observed in BALB/c mice. Oral pretreatment with DF also had a strain-dependent effect. For example, BALB/c mice orally pretreated with DF were rendered hyper-responsive to footpad injection with DF; a significant increase in footpad swelling 24 hours post-injection and significant increases in PLN size, weight and cellularity on day 5 post-injection. By contrast, oral pretreatment with DF had no observable effect on PLN reactions in B6 mice or in congenic BALB/c mice where H2d MHC was replaced with H2b. Furthermore, the hyper-responsiveness in wild-type BALB/c mice was associated with a significant increase in the % of CD69+IL-2+ cells in the PLN. Finally, in vitro studies performed on cells isolated from BALB/c mice injected with DF-induced proliferation of splenocytes isolated from BALB/c mice orally treated with DF. Collectively, these results suggest that DF has strain-dependent immunomodulating properties in mice. Specifically, BALB/c mice, but not B6, orally pretreated with DF are hyper-responsive to the immunostimulating effects of DF when challenged in the footpad, which is a response that may be mediated, in part, by sensitized CD8+ T cells reacting to DF-modified antigens in the footpad presented by H2d molecules.

734 EVALUATION OF A SURROGATE ANTIBODY FOR SAFETY TESTING, PHARMACOLOGY OF A MURINE ANTI-CD11A ANTIBODY IN MICE.


Genentech Inc., South San Francisco, CA and Genentech, South San Francisco, CA.

Surrogate antibodies are a potential solution to the limited safety testing possible with humanized monoclonal antibodies with restricted species cross-reactivity. The purpose of this study was to detect potential adverse effects of a chimeric mouse/rat IgG1 anti-mouse CD11a monoclonal antibody (mMu17) on CD11a+ pregnant female mice and development of the embryo and fetus consequent to exposure of the dam through organogenesis. CD11a is a subunit of lymphocyte function associated antigen-1, a leukocyte surface molecule involved in cell-cell interactions important to leukocyte adhesion and activation. MuMu17 is a surrogate antibody for eflazumab, a humanized anti-CD11a antibody in development for the treatment of psoriasis. Previous studies designed to evaluate the surrogate established pharmacological activity of muMu17 at doses ≥3mg/kg. Further, pharmacodynamic comparisons to eflazumab in terms of downmodulation of CD11a expression on T cells indicated that 3mg/kg muMu17 was an appropriate clinically equivalent dose level and was the lowest dose level selected in this development study. Female CD1 mice were administered muMu17 (5, 10 or 30 mg/kg) via subcutaneous (SC) injections once a week beginning on Day 2 of presumed gestation until Day 16. Exposure to muMu17 and the development of anti-muMu17 antibodies were determined. Upon caesarean-sectioning on day 18 of presumed gestation the number and distribution of corpora lutea, live and dead fetuses and placental weight data were observed to be unaffected. Toxicokinetics inferred exposure to muMu17 and there were no detectable antibodies to muMu17. There were no muMu17 treatment-related effects on the examined parameters including no soft tissue or skeletal malformations. In conclusion, administration of muMu17 to pregnant mice was not associated with any adverse developmental effects.

735 IMMUNOCOMPATIBILITY OF INHALED MURINE CpG OLIQNUCLEOTIDES AS A MUCOSAL ADJUVANT IN COMBINATION WITH RECOMBINANT SEB VACCINE.


Toxicology and Anaesthesiology, USAMRMD, Frederick, MD; Pathology, USAMRMD, Frederick, MD and Coyle Pharmaceutical Group, Wellingt, MD.

Staphylococcal enterotoxin B (SEB) is an exotoxin derived from the bacterium Staphylococcus aureus. SEB, classified as a superantigen and a biological threat agent, is a known inducer of T-cells and can cause illness and death in humans. A USAMRMD recombinant SEB vaccine (rSEBv) protected mice and primates against aerosolized SEB when the vaccine was administered with alhydrogel as an intramuscular (IM) injection. We tested whether administering rSEBv by inhalation would induce mucosal immunity, thus protecting against lethality while also blocking the enteric effects of SEB intoxication. A murine CpG-containing oligonucleotide (CpGp) was used as a mucosal adjuvant in conjunction with rSEBv. Mice were dosed with either rSEBv + CpGp (5 μg each), CpGp alone (5 μg), or saline (10 μl). Administration of 20 μg rSEBv as a result of IM injection resulted in three times the boost vaccination schedule. The use of CpGp alone or with rSEBv induced a transient neutrophil influx three hours post vaccination with no resulting notable

736 APPLICATION OF A SURROGATE ANTIBODY FOR SAFETY TESTING, DEVELOPMENTAL TOXICITY STUDY OF A MURINE ANTI-CD11A ANTIBODY IN MICE.


Genentech Inc., South San Francisco, CA and Argus Research Laboratories, Horsham, PA.

Surrogate antibodies are a potential solution to the limited safety testing possible with humanized monoclonal antibodies with restricted species cross-reactivity. The purpose of this study was to detect potential adverse effects of a chimeric mouse/rat IgG1 anti-mouse CD11a monoclonal antibody (muMu17) on CD11a+ pregnant female mice and development of the embryo and fetus consequent to exposure of the dam through organogenesis. CD11a is a subunit of lymphocyte function associated antigen-1, a leukocyte surface molecule involved in cell-cell interactions important to leukocyte adhesion and activation. MuMu17 is a surrogate antibody for eflazumab, a humanized anti-CD11a antibody in development for the treatment of psoriasis. Previous studies designed to evaluate the surrogate established pharmacological activity of muMu17 at doses ≥3mg/kg. Further, pharmacodynamic comparisons to eflazumab in terms of downmodulation of CD11a expression on T cells indicated that 3mg/kg muMu17 was an appropriate clinically equivalent dose level and was the lowest dose level selected in this development study. Female CD1 mice were administered muMu17 (5, 10 or 30 mg/kg) via subcutaneous (SC) injections once a week beginning on Day 2 of presumed gestation until Day 16. Exposure to muMu17 and the development of anti-muMu17 antibodies were determined. Upon caesarean-sectioning on day 18 of presumed gestation the number and distribution of corpora lutea, live and dead fetuses and placental weight data were observed to be unaffected. Toxicokinetics inferred exposure to muMu17 and there were no detectable antibodies to muMu17. There were no muMu17 treatment-related effects on the examined parameters including no soft tissue or skeletal malformations. In conclusion, administration of muMu17 to pregnant mice was not associated with any adverse developmental effects.

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pathologic sequelae. Immune response was evident in the rSEB + CpG group characterized by increased serum anti-SEB IgG and IgA in lavage. Pulmonary gene expression profile of mRNA species determined by cDNA array analysis indicated 67 upregulated and six downregulated genes in CpG-treated groups. Analysis of splenic lymphocytes had six times the CD4+ T-cells in the rSEB + CpG group compared to the rSEB or sham groups. All groups were then challenged with SEB by aerosol. Survival rates in mice vaccinated by inhalation (92%) were comparable to the mice who were injected (90%) whereas all sham-vaccinated animals died (0%). The results of this study suggest that CpG has the potential to be used as a safe and immunostimulatory mucosal adjuvant when delivered by inhalation.

738 CHARACTERIZATION OF A POTENTIAL ANIMAL MODEL OF AN IDIOSYNCRATIC DRUG REACTION: NEVIRAPINE-INDUCED SKIN RASH IN THE RAT. J. M. Shenton and J. P. Uetrecht. Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada.

RATIONALE: Idiosyncratic drug reactions are difficult to study in humans due to their unpredictability. Unfortunately, this characteristic also hinders the development of animal models needed for mechanistic studies. Nevirapine (NVP), used to treat AIDS, results in a severe idiosyncratic skin rash in some HIV patients. We found that NVP can also cause a significant rash in some strains of rats. METH-ODS: Female rats, Lewis (n=6), Brown Norway (BN, n=12), Sprague-Dawley (SD; n=26) and male rats, BN (n=2), were administered NVP, 100-150 mg/kg/day, in their diet. Animals were monitored for development of skin rash, food intake and body weight. At either week 8 of treatment or after development of rash, animals were sacrificed and blood and skin samples acquired. Some animals presenting with skin rash were removed from drug, allowed to recover and then rechallenged. To determine the effects of lower doses of NVP, female BN rats were treated with 50-75 mg/kg/day (n=4) or 25-40 mg/kg/day (n=4). RESULTS: NVP treatment-induced skin rash in 7/26 female SD rats after 4-6 weeks of dosing and in all female BN rats after 3 weeks of dosing; however, male BN rats, female Lewis rats, and female BN rats treated with lower doses of NVP were not affected. The skin rash consisted of small, red, itchy lesions primarily on the back. Light microscopy revealed a mixed inflammatory infiltrate. Hematology and chemistry tests showed no consistent changes in treated animals. Animals rechallenged with NVP developed skin symptoms faster on reexposure, but the syndrome was different. Instead of presenting primarily with skin lesions, the animals exhibited excessive hair loss commencing at approximately 24 hours post reexposure, occasional skin lesions and general malaise that necessitated sacrifice after less than 2 weeks of treatment. By light microscopy the hair loss was characterized by a perifollicular inflammatory infiltrate. CONCLUSIONS: NVP-induced skin rash in rats may represent a new animal model for the study of drug-induced idiosyncratic skin reactions.


Socio-economic status has been generally accepted as a primary predictor of susceptibility to Sudden Infant Death Syndrome (SIDS). However, recent evidence suggests that other more influential factors may be associated with SIDS, in particular and infant mortality (IM), in general. A computerized data file of matched birth and death tapes, to one year of age (1995-1998) for District 19 Virginia was obtained from the Division of Vital Records, Virginia Department of Health. The fetal deaths were matched for address and zip code at death in addition to birth weight, race and underlying causes of death. The International Classification of Diseases 9th Revision Clinical Modification (ICD 9 CM) was used to numerically code causes of death. The deaths of eighty-seven (87) infants between 1995 and 1998 in District 19 (a given geographical area of Virginia) were examined to determine if any relationship existed for infant death and selected environmental conditions. Fifty-six (56) of these infants were black and twenty-nine (29) were white (64.4% and 33.3% respectively). The majority of these deaths (40.2%) occurred in one geographic area in Petersburg area code 23803 followed by the area Hopewell City, Emporia and Petersburg 23805 (16.1%, 12.6% and 9.2% respectively). The majority of the Petersburg deaths (75%) were black infants. The suspected leading causes of death in this study were due to congenital anomalies (49%) followed by cardio-respiratory conditions including SIDS (26%) and low birth-weight (14%). Other all conditions, as coded by ICD 9 categories, accounted for less than 3% of the deaths. Geographical imaging analysis of District 19 revealed that several neurotoxins were present in areas where IM was highest. Other IM was lowest. Other IM was highest. Other Danges in Virginia, where IM is relatively high, show similar results. As such, there may be a need to examine the risk factors associated with IM particularly among African American women and others disproportionately exposed to selected environmental conditions. Supported by UNGF/FPA/PEJER VU 2001


California's legislature enacted the Children's Environmental Health Protection Act requiring Cal/EPA to specifically consider infants and children in setting criteria for toxic air contaminants (TAC) including cancer potency factors and reference exposure levels. The law mandated the Agency to consider exposure patterns of infants and children, special susceptibility, effects of exposure to more than one TAC with a common mechanism of action, and interactions of TAC and criteria air pollutants in assessing risks to children. The law also requires re-evaluation of the criteria for toxic air contaminants over the next several years to ensure adequate protection of infants and children, and establishment of an initial list of up to 5 TAC that may disproportionately impact children by July 1, 2001. Cal/EPA prioritized the TAC based on known toxicity and exposure. We conducted a focused literature review on 37 TAC looking for specific information pertinent to infants and children's exposure and potential differential response relative to adults. We focused on chemicals associated with developmental, neuro-, respiratory, endocrine, or immune toxicity, and endocrine disruptors. We considered asthma induction and exacerbation as disproportionately impacting children. We considered whether existing criteria were adequate to protect infants and children. After public and peer review by a UC Panel, five TAC were chosen to constitute the initial list: acrolein, polychlorinated dibenzo-p-dioxins and dibenzofurans, lead, polycyclic organic matter, and particulate matter from diesel-fueled engines. This paper describes the results of our deliberations on decision-making criteria and reasons for listing these five chemicals. In addition, we describe efforts to evaluate the adequacy of our risk assessment methods to protect infants and children.

741 POTENTIAL IMPACT OF ENVIRONMENTAL EXPOSURES TO POLYCYCLIC ORGANIC MATERIAL (POM) ON CHILDREN'S HEALTH. A. G. Salmon and T. Meenan. Office of Environmental Health Hazard Assessment, California EPA, Oakland, CA and Biotechnology and SBIR Consulting, Tiburon, CA. Sponsor: E. Zeize.

POM, a class of compounds including polycyclic aromatic hydrocarbons (PAHs) and derivatives, e.g. polynuclear aromatic hydrocarbons and nitro-PAHs, is listed as a California Toxic Air Contaminant. Sources include various industrial, domestic, and mobile combustion processes. In support of the Children's Environmental Health Protection Act (California SB25), we reviewed POM and PAH toxicity to identify hazards to which infants and children might be especially sensitive. Health standards for POM are typically based on carcinogenicity. Many PAHs are animal carcinogens by various routes. PAH-containing mixtures, such as tobacco smoke, coke-oven fumes, and diesel exhausts, are known human carcinogens. Theoretically, from both Armitage-Doll and Moolgavkar models, carcinogenesis exposures early in life lead to greater lifetime cancer risk than later exposures. Experiments have also shown that fetal rat embryos are more sensitive to carcinogenesis by PAHs and PAH derivatives than adults. Studies on pregnant rodents have revealed less widely recognized effects of PAHs, including intratumor growth retardation (IUGR), fetal mortality, and teratogenesis. Recent studies of populations exposed to industrial air pollution have confirmed the sensitivity of the human fetus to PAH-induced IUGR and dysmorphogenesis. PAHs are toxic to the immune, hematopoietic, and reproductive systems. The toxicity to adults is mostly reversible and at high doses, but the fetus or neonate may show severe and lasting effects at lower doses. Both cancer and non-cancer toxicity of PAHs generally correlate with the formation of protein (and especially) DNA adducts by reaction with metabolically generated reactive intermediates. Adducts and metabolites have been used as biomarkers of PAH exposure and adverse effect in humans exposed to air pollution and environmental tobacco smoke. Apparently, children may be both more heavily exposed and also more sensitive to the toxic effects of POM.


As infants developing so quickly might be very strongly affected by various environmental circumstances; it is very important to examine the toxicological profiles and levels of chemicals in newborn to lactating animals and compare them with test results using young animals. For the comparative study, we selected 14 phenolic derivatives, of which 4-nitrophenol and 2, 4-dinitrophenol were chosen for evaluation.
as the first trial. In the 18-day repeated dose newborn rat study, 4-nitrophenol was orally given from day 4 to 21 after birth but did not induce any toxicity up to 160 mg/kg, although it induced death in 10% of males at 160 mg/kg and 3 of 6 males and one of six females at 230 mg/kg in the dose-finding study. In the 28-day repeated dose oral toxicity study starting at 6 weeks of age, 4-nitrophenol caused death of most males and females at 1,000 mg/kg but was not toxic at 400 mg/kg except for male rat renal toxicity. Unequivocally toxic levels of 4-nitrophenol were considered to be 230 mg/kg/day in newborns and 600 to 850 mg/kg/day in young rats. The NOAELs were 110 mg/kg/day in newborn rats and 400 mg/kg/day in young rats. In the newborn rat study of 2,4-dinitrophenol, significant lowering of body and organ weights was observed at 20 mg/kg and animals died at 30 mg/kg in the dose-finding study. In the 28-day young rat study, clear toxic signs followed by death occurred at 80 mg/kg but there was no definitive toxicity at 20 mg/kg. Unequivocally toxic levels and NOAELs of 2,4-dinitrophenol were considered to be 30 and 10 mg/kg/day in newborn rats, and 80 and 20 mg/kg/day in young rats, respectively. Based on these results, it can be concluded that the toxic response of these chemicals in newborn rats is at most 4 times higher than that in young rats.

743 EFFECTS OF PENTYL ETHER, 1, 4-DIETHOXYBUTANE AND 1, 6-DIMETHOXYMETHANE ON MALE RATS FOLLOWING 4-WEEK ORAL ADMINISTRATION.

R. Poon, A. Yagminas and L. Cha, Environmental Health Science Bureau, Health Canada, Ottawa, ON, Canada.

Monomers such as pentyl ether (PE), and diethers such as 1, 4-diethoxybutane (DEB) and 1, 6-dimethoxymethane (DMH) have been proposed as cetane boosters for diesel fuels. The present study was conducted to assess the short-term oral toxicity of these compounds. Male Sprague-Dawley rats (286 +/- 48 g) were divided into 10 groups of seven animals each. The diets were dissolved in corn oil and administered by gavage to the animals at dose levels of 2, 20 and 200 mg/kg body weight/day, 5 days per week for 4 weeks. Control animals received corn oil only. No clinical signs of toxicity were observed throughout the treatment period. No significant treatment effects were detected in food consumption and body weight gain. At termination, animals receiving 200 mg/kg of DMH showed marked reduction in the weight of the testis (30%) and thymus (46%) when compared to the control group (p<0.05). Changes in serum chemistry and histology were also observed in the 200 mg/kg DMH group only, and consisted of decreased serum uric acid level and lacratic dehydrogenase activity, and a 40% decrease in platelet count. No treatment-related changes were detected in N-acetylglucosaminidase activity and protein level in the urine and in the cell-free bronchoalveolar lavage fluid. These results suggest that DMH is more toxic than DEB and PE. Its toxicity in male rats is characterized by testicular and thymic atrophy, and thrombocytopenia.

746 EVALUATION OF HUMAN DEVELOPMENTAL TOXICITY FOLLOWING PESTICIDE EXPOSURE.

S. A. Lacson1, S. A. Tabacova1 and C. A. Kimmel1. 1NCTR, USFDA, Rockville, MD and 1National Center for Environmental Assessment, USEPA, Washington, DC.

Federal regulations require that pesticides be tested for reproductive and developmental toxicity in animals prior to registration to establish limits of exposure. Animal data are qualitatively believed to be predictive of human developmental toxicity, but direct comparisons are limited by the availability of adequate human data for specific chemicals. In this study, we identified pesticides with published human reproductive and developmental toxicity data and selected pentachlorophenol (PCP), a chlorophenolate pesticide, for evaluation based on the number and type of human studies. A total of 7 epidemiological studies or reports of PCP exposure were identified, including 1 cross-sectional study, 3 case-control studies, 2 cohort studies, and a report of 2 cases. The data were extracted into a database and evaluated for reliability using uniform criteria. Overall, studies were of reliable design, excepting the case reports. Four studies included measurements of PCP in tissues or in environmental samples (indoor air). There was a strong effort in most studies to control for confounders of exposure-outcome relationships. Potential reproductive and developmental outcomes of PCP exposure included reduced birth weight and length, and increased risk of spontaneous abortion, pregnancy complications, altered female hormone levels, congenital anomalies (cataracts), and childhood brain cancer. There appears to be reasonable human evidence that elevated PCP levels are associated with adverse reproductive and developmental outcomes. Existing animal data from standard testing batteries will be used to evaluate the predictability for the observed human effects. Supported in part by an appointment (SAL) to the Postgraduate Research Participation Program at the NCTR administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the USDE and the USFDA. The opinions expressed here are those of the authors and do not necessarily reflect the views or policies of the USEPA or the USFDA.

747 PHYSIOLOGICALLY BASED PHARMACOKINETIC ANALYSIS OF PECHLORAL (ClO₄⁻) DOSIMETRICS.


Petchlor (ClO₄⁻) has been found as a contaminant in surface and ground waters of many environmental sites, especially in the western United States. Due to the proven effectiveness of ClO₄⁻ to cause inhibition of iodide uptake by the thyroid gland of mammals, concern has arisen over the potential health consequences to humans consuming contaminated waters. In order to help quantify the potential risk to humans, physiologically based pharmacokinetic (PBPK) models have been developed to calculate the absorption, distribution, metabolism and excretion of
STATISTICAL APPROACH TO BRAIN MORPHOMETRY DATA FROM DEVELOPMENTAL NEUROTOXICITY (DNT) TESTING GUIDELINES: PROFILE ANALYSIS.

A. M. Gelles and A. M. Jarabek, ORD/NHEERL/NT, USEPA, Research Triangle Park, NC and ORD/NHEERL, USEPA, Research Triangle Park, NC.

Brain morphometry measurements are required in test guidelines proposed by the USEPA to screen chemicals for developmental neurotoxicity. Because the DNT is a screening battery, the analysis of this data should be sensitive to dose-related changes in the pattern of brain growth without requiring prior expectations about specific regions or the magnitude and direction of these changes. In addition, analysis should take into account the lack of independence between multiple brain region measurements taken within the same animal, since the sizes of different regions are likely related. Analysis with t-tests or univariate analyses of variance is inappropriate because it assumes independence and runs the risk of Type I error due to repeated sampling using the same error term. A multivariate analysis of variance, profile analysis, was adopted to examine brain morphometry data submitted for the risk assessment of perchlorate (CIO4-), an endocrine-disrupting compound shown to reduce thyroid hormone levels and alter brain development. Profile analysis reduces the number of main effects tested by making between-groups (dose group) comparisons using vectors that comprise all of the within-subjects morphometric data from each animal, then comparing these vectors. It also allows gender to be nested within litter, which is necessary to address DNT study designs. The primary test of profile analysis examines whether the pattern made by the various measurements is similar for all treatment groups. This is a test of the interaction between the treatment factor and the within-subjects scores. If the profiles are not parallel, an interaction exists, and further analyses of the interaction factors may be made to elucidate the effects. Results of this analysis showed that CIO4- affected the pattern of brain growth in developing pups at all doses tested. We conclude that this analysis and observations on profile DNT are important in understanding DNT studies. This is an abstract of a proposed presentation and does not necessarily reflect ESA policy.

QUANTIFYING UNCERTAINTY IN HUMAN REPRODUCTIVE RISK PROJECTED FROM RAT INHALATION STUDIES ON ETHYLENE OXIDE: A FRAMEWORK BASED ON DATA-BASED STATISTICAL DISTRIBUTIONS OF EXTRAPOLATION FACTORS.


Current noncancer risk assessment does not estimate risk as such but instead computes “safe” doses for the general human population, despite its variation in susceptibility. To account for this variation, and for uncertainty in the extrapolation from animals, "Uncertainty Factors" are applied to animal no-effect levels. Resulting "safe dose" estimates are conservative, but their statistical reliability and the protection actually afforded are poorly characterized. Risks (if any) at exposures above the conservative "safe" levels cannot be addressed, hampering rational risk management analyses. We propose an alternative analytical framework in which the specific sources of extrapolation uncertainty and inter-human variability are characterized as statistical distributions based not on policy choices but on actual data for the observed patterns of variation among cases in instances where they have been characterized. We separately propagate the distributions of extrapolation uncertainties and of human variability to estimate a dose-response curve for humans with empirically supported characterization of the uncertainty in dose levels leading to various levels of human population risk (c.g., 1/106, 1/1000). This allows characterization of the reliability of regulatory levels as "safe" doses and permits estimates of the likelihood of impacts (and lack of impacts) on individuals exposed above such regulatory levels. Using a rich database on the reproductive and developmental toxicity of ethylene oxide in rats, a probabilistic characterization of possible regulatory inhalation thresholds is presented as an example of this dose-response based distributional framework. Our method can be applied to comparative risk analysis and benefit-cost analysis, and it furthers harmonization assessment of carcinogens.

GENETIC POLYMORPHISMS IN ASSESSING INTER-INDIVIDUAL VARIABILITY IN DELIVERED DOSE.

J. T. Haber, A. Majer, P. R. Gentry, H. J. Clewell and L. D. Dowsen, TERA, Cincinnati, OH; ICF Consulting, Reston, VA and Environ, Reston, VA.

Genetic polymorphisms have been suggested as an important contributor to overall human variability. Recently, these data have been integrated with pharmologically-based pharmacokinetic modeling as an approach for determining overall variability. We present an analysis of the potential contribution of polymorphisms in enzymes modulating the disposition of four diverse compounds: warfarin, prothrombin, methylene chloride, and dichloroacetic acid. Through these case studies, we identify key uncertainties likely to be encountered in the use of polymorphism data, and highlight potential simplifying assumptions that might be required to test the hypothesis that genetic factors are a substantive source of human variability in susceptibility to environmental toxicants. These uncertainties include (1) the relative contribution of multiple enzyme systems, (2) the extent of induction/inhibition through co-exposure, (3) allelic frequencies of major ethnic groups, (4) the absence of chemically-specific phenotype data, (5) large numbers of low-frequency alleles, and (6) uncertainty regarding differences between in vitro and in vivo kinetic data. Our efforts set the stage for further integration of polymorphism data with PBPK modeling as a means to quantitate population variability.

RELATIONSHIP BETWEEN TOXICITY VALUES FOR THE HEALTHY SUBPOPULATION AND THE GENERAL POPULATION.


The application of transport and dispersion models for risk assessment and management requires accurate estimates of human toxicity. For chemical warfare agents (CWA), most of the available toxicological data and estimates were generated in support of their offensive battlefield deployment. The available human toxicity data come from a limited segment of the general population (GP): relatively young, fit, 70 kg, male soldiers. The present military CWA toxicity estimates are not suitable for use with the GP because they are framed for male soldiers and do not consider potentially sensitive members of the GP. A method was created to convert a median effect dosage and probit slope from a military (or healthy subgroup) to a GP. Up until now, the conversion of a probit slope to a probit slope has not been rigorously explored. It was assumed that individual susceptibilities can be modeled as a log-normal distribution. Modifications for gender differences were addressed. Two models were developed to mathematically describe subpopulations (SP) within the GP: The Tail Model assumes that the SP consists of all individuals having susceptibilities within a tail of the GP distribution. The Bell Model assumes that the SP has a log-normal distribution within the larger GP distribution. Historical military demographics were used to evaluate the probable size of the HSP: 20 to 30% of the GP. The ratio of the medians and the ratio of the probit slopes between the SP and GP are easily determined as a function of the SP size. The two models displayed similar trends in ratio values. Uncertainty factors (UF) were calculated based upon the medians of the SP and the GP, and the probit slope of the GP UF values from this method were consistent with the results of two previous studies that quantified differences between SPs. Qualitative UF estimates used in establishing proposed AEGIs for CWAs require revision in some cases based upon the results of the present study.

INCORPORATION OF HUMAN INTERINDIVIDUAL ENZYME EXPRESSION AND BIOTRANFORMATION VARIANCE INTO HUMAN HEALTH RISK ASSESSMENTS.


Risk assessments include assumptions about sensitive subpopulations, such as the fraction of the general population that is sensitive and the extent to which biochemical or physiological attributes influence sensitivity. Uncertainty factors (UF) account
have been reported for the formation of hemoglobin adducts following exposure to acrylamide. In an effort to improve risk-based decisions for acrylamide, a PBPK model was developed for acrylamide in the rat using existing data from six published studies. The model provides a good description of the kinetics for acrylamide and its oxidative metabolite, glycidamide. Like many chemicals, uncertainty remains regarding the mode of action by which acrylamide produces its carcinogenic effects. For this reason, Monte Carlo methods were used to perform sensitivity analyses in model predictions for four dose measures, each corresponding to a different assumption for the potential carcinogenic mode of action of acrylamide: (1) genotoxicity; (2) sulfhydryl reactivity; (3) dopamine agonist; and (4) glutathione depletion. The results of the sensitivity analyses indicate that the model results for characterizing metabolism are cytochrome P450 and glutathione-S-transferase (4.5%-5% of variance), and tissue binding (64%-64% of variance) were the most important contributors to variation in dose measures predicted by the model. Although limited human data are available with respect to acrylamide kinetics, the PBPK model presented for acrylamide is useful with respect to (1) organizing existing information on acrylamide kinetics; (2) identifying critical data gaps for risk assessments; and (3) prioritizing the collection of additional data.

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STATISTICAL ANALYSIS OF PHOTOCARCINOCGENESIS STUDIES.

D. F. Moele\textsuperscript{1}, J. J. Chen\textsuperscript{1}, P. C. Howard\textsuperscript{4}, P. D. Forbes\textsuperscript{2}, C. P. Suchmanc\textsuperscript{2} and R. L. Kodele\textsuperscript{2} \textsuperscript{1}NCTR, USDFA, Jefferson, AR and \textsuperscript{2}Argus Research, Charleston, Laboratory, Northam, PA.

The induction of skin cancer in HRA/SKH-1 (1-hr/24) hairless mice has been studied for several decades using xenon-arc generated simulated solar light (SSL). This current is the most accepted surrogate for sunlight induced skin cancer in humans. Photocarcinogenecis experiments have been used to study the influence of chemical on SSL-induced skin cancer, where a chemical may decrease or increase tumor latency, or alter the number of tumors on the mice. We have compared several statistical methods for analyzing the tumor latency and multiplicity in a study where male and female SKH-1 mice were either untreated or treated with cosmetic vehicle, and exposed to either 600 or 1200 RBU/wk of SSL for up to 40 weeks followed by up to 12 weeks of observation. The data were analyzed using a logrank test, negative binomial test, tumor multiplicity test of Duitsos, or an adaptation of the single-induction likelihood ratio test of Kedell and Chen. The likelihood ratio, logrank, and negative binomial tests gave similar results with the data. A conclusion from the likelihood ratio test is that the difference between the groups (600 and 1200 RBU/wk, no vehicle) was due to a difference in tumor latency (time to observation) and not tumor multiplicity (number of induced tumors/mouse), although the number of tumors per mouse actually observed at any given time was much higher in the 1200 RBU/wk group than in the 600 RBU/wk group for both sexes. Monte Carlo simulation was used to determine the accuracy of parameter estimates and to evaluate the type I error rates of the tests. These results demonstrate the utility of these tests in analyzing skin tumor data from similarly designed studies.

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FROM MICE TO MEN, CANCERS ARE NOT CERTAIN AT OLD AGE.

E. Pompei\textsuperscript{1} and R. Wilson\textsuperscript{2} \textsuperscript{1}DEAS and Physics, Harvard University, Cambridge, MA and \textsuperscript{2}Physics, Harvard University, Cambridge, MA.

We have studied age-specific incidence data in humans from SEER (US), Holland, England, Sweden, and California and found that organ site incidences do not continue to increase with age, but tend to peak at about age 80, and reduce thereafter toward zero at age about 100. We have also studied cancer incidence (age-specific mortality for lethal cancers) in mice as a function of age in the EDBI undulated controls study cohort ("megamouse" 2-AAF study), where they have been allowed to live very close to their full natural lifetime, and found that similar turnover in incidence occurs at about age 800 days, or 80% of lifetime. A Beta function model (t = (1 - (a + k x t)/b)), where a, b, k are constants) fits both the mouse data and the human data well. The model suggests that increasing replicative senescence with age might be the cause of the turnover at old age. Limited data from National Toxicology Program mice studies suggest that the cancer age distribution, including the turnover points, may be timed shifted by dietary restriction. Results of a large rat cohort study ("two tons of rats")
Cancer slope factors for the large number of chemicals calculated by two different methodologies and software. In the past, the cancer slope factor has been calculated as the upper 95% confidence limit on the coefficient of the linear term of the multivariate model (i.e. q1) for the extra cancer risk over background. The USEPA draft cancer guidelines, released in 1999, however, prescribe the cancer slope factor as the ratio (r) of the linear extrapolation between a point of departure (POD) from observed data and the origin of the dose-response curve. This POD is usually the lower 95% confidence limit on dose associated with 10% extra risk adjusted for background. This default in the EPA draft guidelines is thought to provide an upper bound estimate on cancer risk and to incorporate experimental variability. However, the extent of overestimation of risk inherent in this approach has often been raised as an issue. In this work, we examined to what extent the two approaches differed in practice by calculating s* and q1* for 102 carcinoenic chemicals from the IRIS database. In all cases, the constrained multivariate model was used to fit the dose-response data using the commercial software Global86 and the EPA Benchmark Dose Software released in 2001. Both softwares obtained identical values of s*. Our results indicate that for 81.4% of the cases, s* is within 0.9 to 1.1 of q1*, and with one exception, the ratio s*/q1* is less than 3.0 for the remaining chemicals. The results validate the argument posed by other authors (Crump and Lowe, 1985; Gaylor et al., 1994) that the two approaches are likely to give nearly the similar results when the multivariate model is used. Our results suggest that for the vast majority of chemicals, the new EPA draft new guidelines for the cancer slope factor appears to be neither over nor under protective of public health in comparison with the traditionally estimated q1*. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the USEPA.

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THEORETICAL MODELS FOR DNA ADDUCT FORMATION AND HPRT MUTATION INDUCTION BY LOW-DOSE ETHYLENE OXIDE EXPOSURE OF MICE AND RATS.

D. M. Walker, V. E. Walket and B. R. Scott. Lacrosse Respiratory Research Institute, Albuquerque, NM.

In order to evaluate the effects of low-dose ethylene oxide (EO) exposures upon the induction of N7-2-hydroxyethylguanine (7-HEG) and Hprr mutations, we characterized previously published data using theoretical models. These adduct and mutant frequency (MF) data were from male B6C3F1 mice and F344 rats exposed by inhalation for 4 weeks (6 h/day, 5 days/week) to 0.3, 10 or 33 ppm EO (Walker et al., Carcinogenesis 20:1787-1792, 1999) or to 40, 1000, or 3000 ppm ethylene (Walker et al., Carcinogenesis 21:1661-1669, 2000). The Dose-Response curves for 7-HEG formation in liver, lung, brain, and spleen were adequately characterized using a power function (FP) model expressed as y=a*x^b, where y represents the concentration of EO and x is the EO-induced DNA damage. A value of 1 corresponds to the linear-to-threshold (LNT) model. The power function (FP) model showed that, for the 7-HEG Dose-Response curves for EO-exposed mice, B > 1; while, for the corresponding curves for EO-exposed rats, B < 1. Thus, use of the LNT model to estimate adduct formation will overestimate the risk for EO-induced DNA damage in mouse tissues, but will underestimate the risk for DNA damage in rat tissues. The Dose-Response curve for EO-induced Hprr MFs in T-lymphocytes from exposed mice has been previously shown to be sub-linear (i.e., to have a lesser effect per unit dose of EO than the exposure concentration was lowered (Walker et al., Mutat. Res. 392:211-222, 1997). We applied a mathematical-based model called NEUTROSE-2O to induced MFs in ethylene/oxygen-exposed mice, and this model predicted that the threshold for the induction of excess mutations (i.e., over background) to be approximately 10 ppm EO. These analyses indicate that, at low doses of EO, the Dose-Response curves for 7-HEG and Hprr MFs in rat and mouse do not follow the LNT model. (Supported in part by the Department of Energy Office of Science and Environmental Management).

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VINYL ACETATE-INDUCED INTRACELLULAR ACIDIFICATION: PROOF OF CONCEPT FOR MODE OF ACTION.

M. S. Bogdanski. DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, DE.

Inhaled vinyl acetate (VA) causes epithelial degeneration, cell proliferation and tumors in the nasal cavity of rats, but not mice. Carboxylesterase (CE) catalyzes hydrolysis of VA to acetaldehyde and acetic acid. Acetaldehyde is further oxidized to acetic acid with a stoichiometric yield of 3 protons. Intracellular acidification is hypothesized to be the critical first step preceding cytotoxicity. The purpose of these experiments was to demonstrate that exposure of CE-containing cells to VA causes reduction in intracellular pH (pHi). Primary rat nasal cavity cells were chosen as a model cell system because they contain CE activity comparable to rat nasal epithelium. Freshly isolated rat hepatocytes were attached to polylysine-coated glass coverslips and loaded with the intracellular dye BCECF-AM. Quantitative fluorescence imaging microscopy was used to monitor pHi in response to various concentrations of vinyl acetate. Concentrations of 10 μM - 900 μM VA produced rapid (~70 sec) decrements in pHi (ΔpHi = 0.35 - 0.52) that were rapidly reversible (~70 sec.) upon removal of VA. Pretreatment of hepatocytes with bis2-nitrophenylphosphate, a specific CE inhibitor attenuated the VA-induced intracellular acidification. These results support a mechanism of VA carcinogenesis involving intracellular acidification, cytotoxicity and cell proliferation, and, together with other quantitative toxicity and dosimetry information, support a margin of exposure-based approach to risk assessment. Supported in part by the Vinyl Acetate Toxicology Group, Inc.
761 STRAIN-SPECIFIC DIFFERENCES IN SUPEROXIDE DISMUTASE AND 8-OXOOGUANOSINE DNA-GLYCOSYLASE CAPACITY OF MALE C57BL/6, BALB/c, AND ICR MICE.

T. Stedefol, F. Cardozo-Peles, G. E. Lazuardo, D. Mosquera, M. Banakis, and R. D. Harbison. 1 Institute for Chemical Research, Kyoto University, Kyoto, Japan. 2 Institute of Agricultural Engineering, Polish Academy of Agricultural Sciences, Zabrze, Poland. 3 Dept. of Environmental Engineering, University of South Florida, Tampa, FL. 4 Institute of Allied Health Sciences, University of South Florida, Tampa, FL.

Regulatory agencies use a variety of methods to estimate the risk to humans from exposures to toxic agents. Many of these methods are based on toxicological data derived from animal models, particularly rodents, and have limitations in their ability to predict human risk. The purpose of this study was to characterize the differences in the enzymatic capacity of key enzymes responsible for the clearing of superoxide anions and the oxidized DNA base 8-hydroxy-2-deoxyguanosine (8-oxoG) in mouse strains. The study was conducted to determine if the activity of superoxide dismutase (SOD) and 8-oxo-guanosine DNA-glycosylase (OGG1) varies among mouse strains and to assess the potential for oxidative DNA damage in mouse strains.

762 TRANSGENIC DATA AS PREDICTORS OF CARCINOGENICITY.


Studies of the tumorigenic potential of chemicals conducted in transgenic species offer several advantages. The transgenic strains carry genetic weaknesses that increase and shorten the time to tumor response thereby lessening cost and time for carcinogenicity assays. At the same time, observations of responses in transgenic animals increase our knowledge of the integrated impact of their genetic defects on the cancer mode of action. However, the limited data from transgenic studies makes it difficult to determine how transgenic responses should be applied in weight of evidence determinations for carcinogenicity. EPA examined the concordance between the results from studies of several transgenic strains and the results from standard bioassays. The findings demonstrate the promise that transgenic data may offer an efficient option for identifying potential carcinogens without the necessity of conducting a bioassay in rodent species. However, it will be important to continue to build a database of transgenic data in order to refine its usefulness as a predictive tool.

763 AREA-UNDER-THE-CURVE (AUC) AS A DOSE MORPHIC RESPONSES FOLLOWING 2, 3, 7, 8-TEHTHACRIBENZODIOXIN (TCDD) EXPOSURE.

A. H. Kim, M. C. Koh, and N. J. Walker. 1 Curriculum in Toxicology, UNC-CH, Chapel Hill, NC. 2 Laboratory of Computational Biology and Risk Analysis, NIEHS, Research Triangle Park, NC.

An underlying basis of risk assessment is that an equivalent risk for a certain dose metric exists that allows for extrapolation between species. To better understand the use of AUC as a dose metric for promiscous responses following TCDD exposure, a novel study was designed using a newly revised physiologically-based pharmacokinetic model in the female Sprague-Dawley rat to obtain similar liver AUCs of TCDD by different patterns of exposure. In the first TCDD-treated group, rats received an administered dose of 100 ng/kg/day TCDD in corn oil for 5 days. In the second TCDD-treated group, rats received a high peak dose averaging 1000 ng/kg/day for the first week followed by a lower TCDD administered dose of 23 ng/kg/day for the remainder of the study. Age-matched control rats received corn oil as a vehicle control. Altered hepatic foci (AHF) positive for the placentoid form of glutathione-S-transferase (GSTT1) enzyme were measured from representative lobes of the liver stained immunohistochemically. Volume fraction, expressed as a percentage, is considered to be the most stable measure of AHC. The median volume fraction was 0.045% in control rats and was significantly elevated in both TCDD-treated groups. However, the volume fraction of GSTT-positive foci was significantly higher in the TCDD group than in the control group. This finding suggests that the peak magnitude of TCDD in liver may play a significant role in addition to AUC in the induction of promiscuous responses by TCDD.

764 DOSE DEPENDENT LIVER REGENERATION FOLLOWING CO-ADMINISTRATION OF CHLOROFORM AND TRICHLOROETHYLENE-INDUCED HEPATOTOXICITY IN MALE SPRAGUE-DAWLEY RATS.

S. S. Anand, S. S. Des, S. N. Murphy, M. M. Murugan, and H. M. Mehendale. Department of Toxicology, College of Pharmacy, The University of Louisiana at Monroe, Monroe, LA.

Chloroform (CHCl3) and trichloroethylene (TCE) are common contaminants present in drinking water and regarded as hepatotoxicants. The aim of this study was to develop a dose-response relationship for CHCl3 + TCE binary mixture (BM) by measuring liver injury and tissue repair and to compare the previous results with CHCl3 and TCE alone. Male Sprague-Dawley rats (250-300 g) were administered with 5-fold dose range of both BM CHCl3 (75, 150, and 300 mg/kg, ip) and TCE (1250, 2500, and 5000 mg/kg, ip) for 7 days. Liver injury was assessed by plasma ALT and AST activities and tissue repair was measured by 3H-thymidine incorporation into hepatocellular DNA. Since both these compounds are known to cause hepatotoxicity, blood urea nitrogen (BUN) levels were assayed to evaluate kidney damage. Levels of both the parent compounds in TCE and trichloroethanol (TCH) were quantified by gas chromatography. The levels of CHCl3 peaked in the liver and blood at 60 min and declined thereafter. CHCl3 levels were higher at injection of BM compared to injection of CHCl3 alone. Liver injury, as assessed by ALT and AST, was higher at earlier time points and subsequently reversed. However, dose-response was not evident, possibly due to the inhibition of these transaminases by TCA and TCH, metabolites of TCE. Tissue repair response followed a dose-dependent increase at all time points, and was evident at 24 h and peaked at 48 h. At 24 h, only the highest combination caused kidney damage. The CHCl3 + TCE BM caused either additive or no more than additive hepatotoxicity. This, the present results support the concept that tissue repair response is critical in restraining the progression of injury supported by ATSDR UHEATD 681482.

765 CHRONIC RISK ASSESSMENT FOR TRIBUTYL PHOSPHATE (CAS NO. 126-73-8).

A. R. McDonald, M. Odin, and H. Choudhury. Environmenal Science Centers, Synthesis Research Corporation, North Sidney, NY. and NCEA, ORR, USEPA, Cincinnati, OH.

TrIBUTYL PHOSPHATE (TBP) is a non-flammable, non-explosive liquid that is used as a solvent or anti-foaming agent. Dietary exposure to tributyl phosphate at high doses produces a reversible hyperplasia of the urinary bladder epithelium which is not due to the presence of crystalluria, urinary precipitates or calculi, but may possibly be attributed to the presence of a toxic metabolite(s), tributyl alcohol and dibutyl hydrogen phosphate, which is suggestive of a non-genotoxic mechanism of carcinogenesis. Therefore, several approaches were used when calculating a quantitative estimate of carcinogenic risk. Provisional oral slope factors were derived using both a linearized multistage model and a margin of exposure analysis, and the results produced by the different methods were compared. (This abstract does not necessarily reflect EPA policy).
PROPOSED ORAL SLOPE FACTOR FOR P-CHLOROANILINE UTILIZING LINEAR AND NON-LINEAR APPROACHES.


A review of the available carcinogenicity data for p-chloroaniline identified the chemical as a candidate for analysis of carcinogenic risk by both the traditional linear method as well as the proposed nonlinear (margin of exposure) method. Although no human carcinogenic studies have been published, the available rodent oral studies in rats and mice provide evidence of multi-organ carcinogenicity. The cancer risk assessment for p-chloroaniline is complicated by species differences in target organ susceptibility and uncertainty as to which of the compound's possible carcinogenic mechanisms is most relevant to humans. There is evidence for genotoxic and non-genotoxic modes of action, both of which apparently require bioactivation of p-chloroaniline. The primary tumor types observed in the oral studies are rare splenic tumors (fibrosarcoma, osteosarcoma, or hemangiosarcoma) and adrenal medullary pheochromocytomas in male rats, and hepatocellular carcinomas in male mice. Data on tissue-specific distributions of reductase activities, which protect against the reductive activation of p-chloroaniline, suggest that the mouse hepatocellular tumors may not be relevant to humans. However, either of the rat tumors could be relevant to humans: the adrenal tumors induced by a genotoxic mechanism, and the splenic tumors induced by a non-genotoxic mechanism secondary to erythrocyte toxicity. Dose-response modeling of the incidence data for rat tumors was performed using both linear and non-linear methods. In deriving oral slope factors based on rat adrenal and splenic tumors, the default approach assuming linearity was compared with the margin of exposure approach, which assumes non-linearity. The margin of exposure approach for splenic sarcomas was based on the assumption that an oral dose of p-chloroaniline that was protective for splenic fibrosis (a non-neoplastic lesion) would also be protective for carcinogenicity in the spleen.

ORAL SLOPE FACTOR FOR BUTYL BENZYL PHthalATE (BBP): COMPARISON OF A LINEAR AND NONLINEAR APPROACH.


A review of the available carcinogenicity data for BBP (CASRN 85-68-7) identified the chemical as a candidate for analysis of carcinogenic risk by both the traditional linear method as well as the proposed nonlinear (margin of exposure) method. Currently, BBP is classified as Group C (Possible Human Carcinogen) based on no human data and limited animal data. The basis for this classification is a 1982 NTP study wherein a statistically significant increase in mononuclear cell leukemia was reported in female rats following two years of exposure to 12,000 ppm of BBP in the feed. A quantitative estimate of carcinogenic risk from oral exposure is not currently included on IRIS due to the qualitative weakness of the response, including similar pathologies in the control and treated groups and lack of reduction in time to first tumor. More recently, NTP conducted additional long-term feeding studies with BBP in F344 rats. The results of these studies provided some evidence of an increase in the incidence of pancreatic cancer in male F344 rats, though a significant increase was only seen at the highest oral dose level (12,000 ppm). Concurreal with these neoplastic lesions, the study reported a dose-dependent increase in pre-neoplastic lesions (acinar hyperplasia) in the pancreas. The model(s) of action of BBP-induced carcinogenesis are not clear. There is limited evidence supporting both genotoxic and non-genotoxic mechanisms. The majority of data from genotoxicity testing indicated that BBP is not strongly genotoxic. Moreover, BBP, like other phthalate diesters, induces peroxisome proliferation, which is the putative mechanism of action for the observed changes in the pancreas. A comparison of the results using the default linear approach and a nonlinear (margin of exposure) approach provides insight into the effect of linearity assumptions in deriving of quantitatively estimated oral carcinogenic risk for BBP.

ASSESSMENT OF "ALL CANCERS" IN DIOXIN EPIDEMIOLOGY STUDIES.


The carcinogenicity of dioxin in humans is a source of vigorous scientific debate. As a result, different organizations classify its carcinogenicity differently. For example, USEPA recently recommended that dioxin be classified as a human carcinogen. In contrast, the majority of members on USEPA’s Science Advisory Board’s Dioxin Reassessment Review Subcommittee did not support the classification of TCDD as a human carcinogen. It is therefore useful to compare results of various epidemiology studies, which evaluated the cancer incidences/mortality in populations exposed to relatively large concentrations of dioxin. We selected the epidemiology studies, based on size of population, length of followup, and characterization of exposure, and summarized their findings for the category "all cancers." These studies involved nine occupational cohorts from a number of countries including the US, the Netherlands, and Germany, and one residential cohort in Italy (Sesto). We restricted our analysis to "all cancers," the cancer increase relied on by USEPA as the basis for designating dioxin as a carcinogen. The results show that the relative risks are small or nonexistent even after accidents associated with high exposure levels. For example, in Bertazzi et al. (2001), a study of residents exposed to dioxin in Sesto, Italy, "all cancers" are not increased in the zone A (highest exposed) population in either men and women combined, or in women or men separately (overall or for any time interval since first exposure). In occupational studies, the relative risks are relatively small and mixed; Becker et al. (1996), in a study of four occupational cohorts, found a significant increase in only one of the four cohorts. The increase was relatively small (standardized mortality ratio (SMR) = 134), and was
found in the cohort with the shortest mean duration of employment. Therefore we conclude that, based on epidemiology studies, the evidence for an association between diosin and excess total cancer in humans is mixed, at best.

771 ASSESSMENT OF BENZENE CARCINOGENIC POTENTIAL IN HUMANS.

Benzene is currently classified as a human carcinogen based primarily on increased risks of leukemia, with acute myeloid leukemia as the predominant form. Although some researchers have suggested that benzene also causes other types of cancer such as lymphoma, non-Hodgkin's lymphoma, multiple myeloma, brain, lung, and kidney cancers, it is of interest to assess the evidence for the carcinogenicity of benzene with respect to these other tumor sites. We selected seven epidemiological studies determined to be of sufficient quality, based on analysis of specific cancer outcomes, assessment of benzene exposure, and cohort size of more than 300 workers. These studies involved cohort studies of workers in painting, printing, painting, and dry cleaning, chemical production, oil refineries, and workers as car and mobile equipment mechanics, and filling station managers in China, Italy, Sweden, the United Kingdom, and United States. While excess lymphoma, non-Hodgkin's lymphoma, and lung cancer were observed in a group of benzene-exposed Chinese workers, five other studies did not demonstrate any significant increase in risk among other workers exposed to benzene or benzene-containing petroleum products. Findings in multiple myeloma, brain and kidney cancers in workers were also inconclusive, for example, there was significant increased risk of brain cancer in small service filling stations, but not in large service filling stations, and two other studies did not demonstrate any significant increased risk of brain cancer in workers exposed to benzene. Therefore, we conclude that there is no convincing evidence for an association between benzene and the tumor types noted above.

772 AN ASSESSMENT OF THE HUMAN RISKS ASSOCIATED WITH RADIOFREQUENCY EXPOSURE.

Many emerging technologies depend on radiofrequency (RF) electromagnetic (EM) energy. Consequently, public concern regarding potential hazards associated with RF exposure is high. To assess the human health effects of low-level RF exposure, 80 epidemiology studies published since 1965 related to EM radiation exposure were identified; these were categorized according to RF frequency ranges (0-300 kHz, 0.3-30 MHz, and 0.3-300 MHz). The majority of studies identified dealt with cancer endpoints. Within each EM frequency category, studies were further classified according to study design (cohort, case-control, etc.). Studies of exposures in the 0.3-300 MHz range (the RF range) were given greater weight in assessing potential health risks. These included: ecologic studies, surveys of RF-exposed workers, case-control studies, and one cross-sectional report. Overall, the individual epidemiology studies related to EM frequency exposures are deficient for risk assessment purposes; they suffer from methodological flaws, design biases, and inadequate exposure measurements. Analyzed as a whole, using the Hill criteria for causation, these studies do not provide evidence of adverse health outcomes related to low-level RF exposure. Those studies that reported potentially increased health risks associated with RF exposure (primarily brain and hematopoietic cancers) failed to demonstrate consistent exposure-response relationships and showed disparate findings that were not confirmed in other studies. The studies related to the 0.3-300 kHz and 0.3-30 MHz frequency ranges also reported discordant findings with inconsistent exposure-response data. The lack of epidemiologic evidence for potentially adverse human health outcomes related to RF exposure is supported by animal data and mechanistic studies which demonstrate that temperature increases in exposed tissues (which do not occur at exposure levels below current US regulatory limits) are required for biological effects. In summary, the available epidemiology data suggest that exposure to low-level RF is not associated with adverse human health effects.

773 LUNG CANCER MORTALITY AMONG WORKERS EXPOSED TO AIRBORNE HEXAVALENT CHROMIUM.

This study assesses mortality through 1997 for 492 persons who worked at a chromate production plant for at least one year between 1940 and 1972. Cumulative airborne Cr(VI) exposures were quantified for each worker and grouped together with this mortality assessment, provide new data for use in cancer risk assessment. Cohort members were followed for mortality through 1997, and standardized mortality ratios (SMRs) were estimated. Lung cancer mortality was investigated further by calculating SMRs stratified by duration of employment, time since hire, year of hire, and categories of cumulative exposure to Cr(VI). Included in this analysis are deaths caused to lung cancer, 303 deaths occurred specifically for all causes (SMR=129, 95% CI 115-144), all cancers (SMR=155, 95% CI 125-191), and lung cancer (SMR=241, 95% CI 180-317). Lung cancer mortality was significantly elevated for the highest two cumulative exposure groups—2.05 to 2.70 mg/m²-years, SMR=365, 95% CI 208-592; and 2.71 to >2.90 mg/m²-years, SMR=3, 95% CI 283-716—but not for the lowest three exposure groups. Significantly elevated SMRs were also found for those hired before 1960, with 20 or more years of employment, and with latency of 20 or more years. No excess lung cancer was found among workers first employed after 1960, which may reflect the transition to a lower-time process and lower exposures due to improved industrial hygiene. The stratified analysis of lung cancer mortality by cumulative exposure suggests a threshold effect, because risk is statistically significantly elevated only at exposure levels over 1.05 mg/m²-years. Although a threshold is consistent with recent published toxicological studies, this finding must be interpreted cautiously, because the data are also consistent with a linear Dose-Response, perhaps due to the small numbers of lung cancer deaths in the lowest exposure groups.

774 DOSE-RESPONSE ASSESSMENT FOR LUNG CANCER MORTALITY OF AN OCCUPATIONAL COHORT EXPOSED TO AIRBORNE HEXAVALENT CHROMIUM.

The dose-response relationship for inhalation exposure to hexavalent chromium (Cr(VI)) and lung cancer mortality is assessed for persons who worked at a chromium production facility from 1940 through 1972. Dose metrics of maximum 8-hour time-weighted-average (TWA) exposure, defined as the highest TWA exposure estimated for each worker, and lifetime cumulative exposure (e.g., mg/m²-years) were evaluated. Poisson and Cox regression models were used to estimate a benchmark dose for 10% additional lifetime risk and unit risks (risk from exposure to 1 µg/m³) for occupational and continuous (e.g., environmental) exposure scenarios. Trend analyses were performed to identify the highest exposure level for which there is statistical evidence of an increased risk of lung cancer. Cumulative exposure to Cr(VI) ranged from 0 to 29 mg/m³-years, and the maximum TWA exposure ranged from 3 µg/m³ to 5.18 µg/m³. A linear dose-response was observed in both of these exposure ranges. The benchmark dose for continuous exposures was 10 µg/m³, and the maximum likelihood estimate of unit risk was 0.011 µg/m³. However, the dose-response was also consistent with a threshold for both dose metrics. There was no statistical evidence of an increased cancer risk at maximum occupational TWA exposures of 16 µg/m³, or cumulative exposures of 0.8 mg/m²-years, corresponding to a 40-year occupational exposure level of 20 µg/m³. The estimated unit risks are approximately equal to those developed by the USEPA in 1998, and are two to six times lower than those proposed by OSHA in 1995. The absence of a linear trend below certain doses suggests a threshold effect although a non-threshold dose-response cannot be ruled out. The existence of an increased cancer risk at low exposure levels is consistent with mechanistic data suggesting that low exposure levels, Cr(VI) is reduced and to detoxified by extra- and inter-cellular components of the lung.

775 USING QSAR IN THE SELECTION OF SURROGATE TOXICITY VALUE.
C. J. Mondal, R. M. Bruce and H. Chandnigarh. NCEA/ORD, USEPA, Cincinnati, OH.

The National Center for Environmental Assessment, Cincinnati (NCEA-Cin), through its Superfund Technical Support Center (STSC), provides rapid-turnaround technical support to Regional staff and others for time-critical decisions making under the Superfund program. A key element of this support is the development of provisional toxicity values, such as oral Reference Doses (RfDs), inhalation Reference Concentrations (RfC), oral slope factors (SFo), or inhalation cancer unit risks (URs). When the published data are inadequate for the development of toxicity values, the Center makes an attempt to identify an appropriate surrogate using a quantitative structure activity relationship (QSAR) approach. A commercial QSAR model, "TOPKAT," is utilized to establish a degree of structural similarity between the query chemical (chemical under examination) and the compounds in the models database. The two models utilized in the selection process are the Oral Rat Chronic LOAEL and the Rat Oral LD₅₀ models. The
query chemical is initially queried using the LOAEL model. Once a valid prediction is obtained (i.e., if all model requirements are satisfactorily met) a "similarity search" is conducted which determines the database compounds most similar to the query compound in terms of common descriptors. All database chemicals that satisfy the similarity distance of 0.200 (distance judged by TOPKAT® models to represent most similar compounds) are considered to be potential surrogates and are then evaluated for toxicity values on IRIS, HEAST or Provisional Toxicity Value databases. If the query chemical does not satisfy all model considerations for the LOAEL model a valid prediction is not obtained and the chemical is queried utilizing the LD50 model to determine a possible surrogate. An example of this selection process will be presented using dichlorobromomethane (DCBM) a metabolite of chloromethane (CB). This abstract does not necessarily reflect EPA policy or endorsement.

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A COMPARISON OF SIX DOSE-RESPONSE MODELS FOR USE IN QUANTITATIVE RISK ASSESSMENT OF WATERBORNE PATHOGENS.

N. A. Niemuth, Z. J. Mal, B. Bouin, and J. C. Lipscomb. Battelle, Columbus, OH and NCEA, USEPA, Cincinnati, OH.

Assessment of risk from waterborne pathogens requires the development of a dose-response model to characterize the relationship between the level of microbial exposure and the probability of a particular adverse consequence (e.g., infection/disease). A properly chosen dose-response model enables us to estimate the individual infectious dose (ID) necessary to cause infection or illness in a percentage of the population. Several models have been used in the literature to describe microbial dose-response data. These include experimental and beta-trend models, which are considered biologically plausible models; log-logistic, log-probit, and Weibull models, which are considered empirical models; and the Weibull-gamma model. There is no consensus on which model is most applicable to microbial dose-response assessment, although the biologically plausible models are favored by many authors. In selecting a dose-response model for microbial risk assessment, we recommend considering the following: (1) goodness-of-fit, (2) simplicity in model formulation, without sacrificing goodness of fit, and (3) conservative prediction of infectious dose at low levels. Each dose-response model was fitted to data collected by Schiff et al. (1984) for echovirus 12 administered to healthy human volunteers in monochlorinated water. The beta-Poisson, log-logistic, log-probit, and Weibull models all provided good fits to the data, with the estimated 1% IDs ranging from 1.7 to 17.0 PFU for these models. The most conservative estimate of 1.7 PFU from the Weibull model was at least four-fold less than doses predicted by the other models, although the bootstrap 95% confidence interval for the 1% ID from the model (0.06, 18.6) included all of the estimated values from the other models. Although not a formal test, this suggests that the model predictions were not substantially different.

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DEVELOPMENT OF A TOXICOLOGY DATABASE FOR THE PREDICTION OF TOXICITY: AN ILSI/HESI STRUCTURE ACTIVITY RELATIONSHIP PROJECT.

P. N. Judson, P. A. Cook, K. A. Stettler, K. V. Thomas and W. J. Brook. ILSI, Ltd., Leeds, United Kingdom; Procure & Gemini, Cincinnati, OH; Health and Environmental Sciences Institute, International, Washington, DC; and Unilever, Edison, NJ, Sponsor: W. Brook.

Scientists need to rapidly predict the toxicity of new compounds using computerized models based on quantitative structure activity relationship (QSAR) or other techniques. The predictive ability of these models will largely depend on the availability of reliable data for sufficient compounds covering a broad array of chemical structures. An international collaboration of the ILSI Health and Environmental Sciences Institute and LHASA at the University of Leeds (UK), has developed a pilot database of toxicity testing data. The collaboration also includes global industrial manufacturing organizations in the consumer products, chemicals, and pharmaceutical sectors, as well as representatives from international regulatory agencies. The pilot database has been populated with toxicity data for four endpoints: carcinogenicity, mutagenicity, dermal sensitization and hepatic toxicity compiled from publicly available sources. The pilot database was derived from the European Chemical Bureau's (ECB) International Uniform Chemical Database (IUCLID) system, and the database has been used for structure or substructure searches and biological data. The database will be further populated with data from additional toxicological endpoints. The database will be made publicly available for development and improvement of predictive toxicological models, and as a screening tool for early chemical hazard identification. The benefits of this project include increased reliability and speed of prediction of toxicity for new chemical moieties, e.g., pharmaceuticals, cosmetic ingredients, etc., and a potential reduction in animal testing.

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STATISTICAL METHODS FOR ANALYZING COMMUNITY INTERVENTION STUDIES: APPROACHES FOR EVALUATING SAMPLES BELOW DETECTION LIMITS.


Interpretation of data below detection limits is always problematic because most methods of statistical analysis assume that all values are observed. When the fraction of samples below a detection limit is 10% or less, the common recommendations to substitute half of the detection limit or 0 for non-detects which allows reasonable use of many statistical methods. When a large fraction of the measurements are below a detection limit a more robust procedure is needed. A number of statistical methods have been developed to analyze this type of data, but often are not easily applied by investigators. We have developed simplified methods for simulating the data below detection limits such that standard methods of statistical analysis can be applied. These methods assume that the shape of the distribution of values above the detection limit provides information about the shape of the distribution below a detection limit through the use of quintile-quintile plots. We applied these methods to analyze data on organophosphorus pesticide metabolites in urine from children in a community intervention study. The pesticide metabolite area had multiple detection limits and groups of metabolites were summed for estimates of exposures. The results illustrate that, in complex situations the proposed method has many advantages in reducing bias compared to the usual recommendations for dealing with measurements below a detection limit, and provides better estimates of confidence intervals. Validation of the new methods through simulation studies showed that the estimation of bias by the proposed method compared to usual methods can be over 90% when non-detects are 25% of the samples, and that adjusted 95% confidence intervals contain the true value 90% to 95% of the time. (Supported by Center for Child Environmental Health Risk Research through grants EPA RB8086601, and NIEHS SP01ES09601-05, and by National Research Center for Statistics and the Environment through grant EPA CR825173.)

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HIERARCHICAL CLUSTER ANALYSIS OF NEUROLOGICALLY RELATED ADVERSE EVENTS ASSOCIATED WITH THERAPEUTIC USE OF DRUGS.

P. S. Pine, E. J. Matthews, J. L. Weaver, J. E. Contessa and J. P. Hangan. Center for Drug Evaluation and Research, USFDA, Laurel, MD.

We extracted neurologically related data from the FDA adverse drug reports (ADR) database for up to the first five years of actual sales for ~335 drugs approved for marketing between 1978 and 1997. The dataset was then limited to drugs that had at least 5 reports for any one of the adverse events and adverse events that had at least 5 reports for any one of the adverse events and adverse events that had at least 5 reports for any one of the adverse events and adverse events that had at least 5 reports for any one of the adverse events. The result was a matrix of 252 drugs versus 114 neurologically related adverse events. Hierarchical cluster analysis of the dataset placed ~20% of the drugs into clusters with correlation coefficients of greater than 0.9. Half of those drugs were found in clusters containing other drugs with similar clinical indications, demonstrating the ability of this approach to detect relationships between drugs. Clusters containing drugs with dissimilar indications may be related by other activities dependent on common structural components. Next we looked at how the adverse event categories related. Cluster analysis placed ~25% of the adverse events into clusters with correlation coefficients of greater than 0.9. The National Library of Medicine’s MeSH (medical subject heading) system was used to evaluate the relationships of the adverse event clusters. Half of the highly correlated events were found in clusters with similar MeSH headings. Combining data for adverse events with similar profiles creates composite event categories that may increase their utility in developing structural alerts. This database combined with structural data for this set of drugs has been used to develop neurotoxicity modules for a quantitative structure-activity relationship expert system.

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CONTRIBUTION OF INTERLEUKIN-10 (IL-10) TO LANGERHANS CELLS MIGRATION IN MICE PROVOKED BY CHEMICAL CONTACT AND RESPIRATORY ALLERGENS.

M. Cumberbatch, R. J. Dearman and J. Kimber. Immunology, Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom.

Following prolonged topical exposure of BALb/c strain mice to chemical contact and respiratory allergens divergent immune responses indicative of preferential Th1 and Th2 cell activation are elicited, respectively. Thus, under conditions of exposure that induce equivalent levels of immunogenicity with respect to lymph node cell (LNC) proliferation and IgE antibody production, only respiratory allergens provoke IgE antibody and a Th2 cytokine secretion profile by LNC.
We have now investigated the contribution of dendritic cells (DC) to the development of these polarized immune responses. We report that topical exposure of mice to contact allergens such as 2,4-dinitrochlorobenzene (DNCB; 1%) and oxazolone (Ox; 0.5%) results in a significant reduction in epidermal Langerhans cells (LC) frequency (20-50%) within 4 hours. In contrast, similar treatment with the re-activated T cell line (YFA-TMA; 2%) failed to provoke such changes in LC densities after 4 hours, although LC values were significantly reduced by 17 hours. Enumeration of DC accumulating in draining lymph nodes (DLN) 18 hours following treatment with these chemicals revealed that in DC numbers induced by DNCB and Ox were 2-fold greater than those observed with exposure to TMA. Similar DC frequencies to those recorded for TMA were provoked by topical treatment with fluorescein isothiocyanate (FITC; 1%), a sensitizing chemical which also induces a Th2-type immune response. Furthermore, following local administration of a neutralizing anti-IL-10 antibody prior to exposure at the same site to chemical, TMA- and FITC-induced DLN DC accumulation was unaffected. These data suggest that chemical contact and respiratory allergens display differential kinetics of LC migration and DC accumulation and that the delayed response observed to these chemicals that induce Th2-type responses may be due to an early inhibitory signal provided by IL-10.

781 MURINE cDNA ARRAY EXPRESSION PROFILING OF CUTANEOUS SULFUR MUSTARD INJURY.


"Medical Research Council of Canada, O.D. Sealy, D.F. Verneris, P.D. Bharadwaj, M.D. DeMarchi, E.D. DeMarchi, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD and Pharmacology Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.

The chemical warfare agent, sulfur mustard (SM) produces skin blister formation with a severe inflammatory reaction in exposed individuals. The mechanism of SM induced tissue injury is not well understood and there is no established medical treatment to counter its toxicity. Our previous studies have identified increases in inflammatory gene expression in both in vivo and in vitro models of vesicular injury. This study examined SM-induced alterations in gene expression in mouse skin using cDNA arrays to identify transcriptional events associated with SM toxicity. Ear skin from 5 mice, paired as SM-exposed (right ear) and unexposed (left ear) from the same animal, at seven exposure doses (5, 10, 20, 40, 60, 80, and 160 mg) was harvested at 24 hours post-exposure. The highest SM dose (160 mg) produced severe injury characterized by edema, dermal infiltration of inflammatory cells, premature death of basal layer epidermal cells, and epidermal-dermal separation. The lowest concentration (5 mg) produced no apparent tissue damage. Screening of 1176 cDNAs that were normalized as exposed/unexposed skin sample from the same mouse identified numerous genes activated or repressed ≥2-fold, with the number of altered genes increasing with SM dose. The mean level of expression (5 animals) for 54 genes increased and 78 genes decreased ≥2-fold at one or more exposure doses when compared to the 5 mg dose. Real-time RT-PCR confirmed the up-regulation of IL-1B and IL-6. The transcriptional activation or repression of a number of genes previously known to be associated with SM toxicity was identified. (Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010)

782 DERMAL PENETRATION OF TESTOSTERONE IN VIVO. PREDICTION FROM SKIN IN VITRO.


Testosterone, the steroid hormone, has been used in topical applications to achieve a systemic effect. Due to its lipophilicity (Pow 3.32) it is efficiently absorbed and has recently been proposed as a marker chemical for predictions of percutaneous penetration using skin in vivo. However there is limited data on direct comparisons of equivalent applications in vivo and in vitro. Whole or dermal forestomach (250 mg) fresh skin from 28 day old male Wistar rats was mounted in Scatchard-Dick Flow-through diffusion cells at 32 C for 24 hrs. Receptor medium (Eagle's Minimal Essential Medium [MEM] supplemented with 2% (w/v) BSA, pH 7.4) was pumped below the skin at 1.5 ml/hr. [14C] Testosterone (10 ml [17 kBq]) was applied to the skin surface (0.64 cm2) at 200 mg/ml in ethanol and receptor fluid samples were collected hourly for 24 hrs. In a parallel in vitro study in four rats 100 ml testosterone was applied to a shaved dorsal area (6.25 cm2) at 200 mg/ml. Lomir dermal jackets protected the backs of the animals for a 24 hr period. Animals were individually housed in metabolism cages from which the excreta are collected. Material in the carcass, urine and feces was determined. In one 12% of the dose was recovered from the skin surface, 2% within the stratum corneum, 3% in the skin at the application site, 18% in urine, 2% in faeces and 40% in carcass.

Therefore 65% of the dose was absorbed beyond the stratum corneum. By comparison, in vivo data indicated that: 35% of the applied dose remained unabosorbed on the skin surface, 3% within the stratum corneum, 7% (1% if dermertanned skin) within the skin and 30% (40% if dertanized skin) was recovered in the receptor medium. In conclusion, low absorption of testosterone in the rat can be reasonably predicted by in vivo model however the underestimated may arise due to limited perfusion of the skin which cannot be modeled adequately. (Supported by SEAC Toxicology Unit. Unilever Research.)

783 NOVEL PEPTIDES PROTECT AGAINST SULFUR MUSTARD-INDUCED SKIN TOXICITY.

U. Worster, B. Broksky and A. Nyska. Faculty of Science, The Hebrew University, Jerusalem, Israel and Laboratory of Experimental Pathology, NIEHS. Research Triangle Park, NC.

Our previous studies demonstrated the protective effect of topical iodine treatment against sulfur mustard (SM)-induced skin lesions. It was proposed that the mechanism involves iodine-induced production of skin protective factors. To test this hypothesis guinea pig skin was exposed to heat followed by treatment with iodine and skin extraction. Intradermal injection of the tissue extract resulted in statistically significant reduction of 60% in SM-induced ulceration area. Two pharmacologically active peptides were identified; partial sequence of histone H2A and fibronectin A; administration of each of these peptides resulted in statistically significant reduction of 67% and 62% respectively, in SM-induced ulceration area. The peptides were coadministered with a cocktail of proteinase inhibitors to prevent degradation. In order to stabilize the peptides we prepared a series of N-methylated peptides that were synthesized and tested in vitro. We propose a new pharmacological approach for whole skin protection, and maybe for whole body protection, against vesicants and other noxious stimuli (supported by USAMRMC Cooperative Agreement No. DAMD17-98-2-0099).

784 UPTAKE AND ACTIVITY OF CASPIAIN AND NONIVAMIDE IN HUMAN SKIN AS A FUNCTION OF VEHICLE.


Caspiain and its analog, nonivamide, are commonly used in the preparation of commercial "pepper sprays" for use as less-than-lethal deterrents. These natural products produce a skin erythema response that can be quantified using the "a" scale colorimeter. A study compared erythema with the uptake of caspiainoids and nonivamide in ventral forearm stratum corneum in seven subjects (47.3 ± 15.7 yrs) as a function of the vehicle: 95/5 (+)/ ethanol/methanol (EtOH), 70/30 (+)/ isopropl alcohol/water (IPA), 80/20 (+)/ mineral oil/isopropyl alcohol (MO) and 80/20 (+)/ propylene glycol/propyl alcohol (PG). Solutions were prepared using 3 mg/ml of capsiain or nonivamide in the above vehicles and applied as a single, unoccluded 5 ml, dose to both ventral forearms of each subject for 10 minutes in a random manner. Caspiainoid concentration was quantified by harvesting stratum corneum from treated sites using twenty 1.3 cm diameter adhesive discs that were subsequently weighed, extracted and analyzed for caspiainoids using LC-MS. Erythema was quantified over 90 minutes after residual product removal on the other arm with a Minolta CR200 colorimeter. The amount of stratum corneum weight harvested from the various sites was independent of the vehicle applied (1.264 mg). The first ten adhesive discs contained the majority of caspiainoids and nonivamide in the skin. EtOH and IPA delivered 3-5X greater amount of caspiainoids to the stratum corneum than MO or PG (p < 0.01). While EtOH delivered 2-5X greater amount of nonivamide than IPA, MO or PG (p < 0.05). EtOH delivered 2X more nonivamide than caspiainoids into stratum corneum, yet area under the a scale-time curve (AUC a) demonstrated similar erythema independent of vehicle. Relative potency (AUC a, concentration) of caspiainoids was 2X greater than nonivamide in EtOH and IPA vehicles, but similar in PG and MO. Thus, vehicle influences both delivery and potency of caspiain and nonivamide in human skin in vivo.

785 TRANSCRIPTOMIC PROFILES IN RAT SKIN FOLLOWING DERMAL EXPOSURE TO SODIUM LAURYL SULFATE.

C. M. Garrett, J. V. Rogers, C. Wang and J. N. McDonald. Biomedical Sciences, Geo-Centers, Inc., Wright Patterson AFB, OH and Marine Environmental Sciences, Inc., Wright Patterson AFB, OH.

Dermal irritation continues to be an important yet poorly understood occupational health issue. Irritant contact dermatitis (ICD), the most common occupational skin disease, costs government and industry millions of dollars each year. Gene array
technology can serve as a tool for assessing chemical irritancy potential. To determine the transcriptional response to dermal irritants, male Fisher 344 rat skins were exposed to 10% sodium laurel sulinate (SLS, a model skin irritant) for 1 hr in vivo. Skin total RNA was isolated at 0 hr (control), and 1 and 4 hrs following the beginning of the exposure to monitor transcription using the Affymetrix U34 array. Our results indicated that dermal exposure to SLS resulted in a significant change of gene expression: more than 20 genes (i.e., inflammatory, Bcl-2-related, oncogenes) were increased >2-fold, whereas more than 24 genes (i.e., cytotoxicity P450-related, tumor suppressor) showed a >2-fold decrease (1 hr vs 0 hr controls). By 4 hr, more than 60 genes (i.e., inflammatory, oxidative and cellular stress, cell cycle, transcription factors, Bcl-2-related) were up-regulated and more than 10 genes (transcription factors, junctional proteins) were down-regulated by a factor >2-fold when compared to the 0 hr controls. A strong temporal change of gene expression was observed in the skin following exposure to SLS. Characterization and analysis of the transcriptomic response of skin to SLS can enhance the risk assessment of dermal irritants.

786 IN VITRO ASSESSMENT OF OXIDATIVE STRESS AND CYTOTOXICITY IN LIVING DERMAL EQUIVALENTS EXPOSED TO M-XYLENE.

J. V. Rogers*, C. A. Coleman, B. E. Hull* and J. N. McDougal*, Geo-Centers, Inc., Wright-Patterson AFB, OH and Wright State University, Dayton, OH.

Dermal exposure to volatile organic chemicals (VOCs) can lead to irritation, inflammation, and cytotoxicity in vivo. Using an improved in vitro exposure method for VOCs, we tested for oxidative stress and cytotoxicity in dermal fibroblasts exposed to m-xylene. Dermal equivalents containing 2.5x10^7 cells were incubated for 1 hr in a m-xylene/cultured medium mixture (range: 0.05 to 11.5 µg/ml m-xylene). Following exposure, cell viability (MTT assay and cellular LDH activity), cellular thiol levels, and endogenous catalase activity were measured. At 1 and 4 hr, cell viability decreased with increasing m-xylene concentration. The EC50 calculated using the MTT assay was 0.35 ± 0.26 and 6.85 ± 0.33 µg m-xylene/g tissue at 1 and 4 hr, respectively. The EC50 at 4 hr was significantly lower (P<0.05) compared to the EC50 calculated from the 1 hr exposure. At 1 hr, the EC50 determined from measuring cellular LDH activity was 23.7 ± 0.88 µg m-xylene/g tissue. By 4 hr, the EC50 had decreased significantly (P<0.05) to 9.24 ± 0.05 µg m-xylene/g tissue. A temporal decrease in the levels of endogenous antioxidants (catalase and thiols) was also observed with increasing m-xylene concentration. Pretreatment of dermal equivalents with the antioxidant N-acetyl-cysteine significantly increased (P<0.05) cell viability. These results suggest oxidative stress may promote m-xylene-induced cytotoxicity in living dermal equivalents. Moreover, these VOC-induced cellular responses could ultimately be related to actual dermal exposure scenarios. (Supported by NIOSH/CDC and AFOSR/NRL)

787 PREDICTION OF MOLECULAR MECHANISM OF SKIN IRRITATION AFTER ACUTE EXPOSURE TO SODIUM LAURYL SULFATE.

R.G. Gunasekara†, J. V. Rogers*, M. B. Kabbur*, W. W. Brinkley*, C. M. Garrette† and J. N. McDougal*,† Pharmacology and Toxicology, Wright State University, Dayton, OH, Geo-Centers, Inc., Wright-Patterson AFB, OH and Operational Toxicology Branch, Air Force Research Laboratory, Wright-Patterson AFB, OH.

Sodium laurel sulfate (SLS) is an anionic surfactant, causing irritation contact dermatitis. Skin irritation by this detergent is not well understood due to the complex molecular interactions. Knowledge of molecular mechanisms and the relationship between duration of surfactant exposures on the skin and the degree of irritation is limited. Here we measured an early inflammatory mediator, interleukin 1-alpha (IL-1α), inducible nitric oxide synthase (iNOS), nitric oxide (NO) and ROS in response to two different concentrations of SLS (1% and 10%)-induced irritation. The dorsal thoracic aspects of male F-344 rats were exposed to SLS for 1 hr using Hill Top Chambers. At 0, 1, 2, 4 and 6 hours after exposure, skin samples were processed for analysis. Western blot of 1% SLS exposed skin samples showed an increase in IL-1α protein levels from 13-37% over controls at various time points, while IL-1α in 10% SLS samples only increased by 10%. This protein induction reached a maximum at 2 hr. The change in iNOS levels in 1% SLS skin over the respective controls was comparable to change in 10% SLS exposed skin samples but occurred at 4 hours. However, NO levels showed a different response. iNOS protein level in 1% SLS exposed samples, NO levels increased peaked at 4 hr after beginning of exposure. In 10% exposed samples NO level peaked at 1 hr after exposure and gradually decreased. There was a significant generation of ROS seen in 1% SLS exposed skin, which reflects the damaging effects on the skin. The reduced level of thiobarbituric acid reactive species (TBARS) in the sample was associated with oxidative stress and skin damage during SLS exposures. These results suggest that the change in the levels of skin's molecular and biological response to SLS exposure explained the biochemical mechanisms associated with SLS-induced irritation (Supported by CDC/NIOSH RO1 OH03654-03).

788 HISTOPATHOLOGIC ASSESSMENT OF ACUTE DERMAL EXPOSURE TO N-METHYL-\(\text{\textasciitilde}{\text{N}}}\)-DIMETHYLAMINE AND SODIUM LAURYL SULFATE IN RATS.

W. W. Brinkley†, J. V. Rogers*, M. B. Kabbur*, C. M. Garrette†, K. T. Geiss†, J. N. Gunasekara† and J. N. McDougal†, AFRI/HEST, Wright-Patterson AFB, OH, GEO-CENTERS, Inc., Wright-Patterson AFB, OH and Wright State University, Dayton, OH.

Skin exposure to organic chemicals and solvents may cause skin irritation and generate proinflammatory mediators that initiate an irritant response. Histopathology can serve as a fixed reference point in a changing microenvironment for explanation of concurrent cellular responses. Changes in the levels of selected proteins in the irritant cascade were quantified after an adjusted time compared to visible tissue changes. Here we describe the dermal pathology of male F-344 rats exposed to met-xylene, dimethamine and sodium laurel sulfate for one hour using Hill Top Chambers. Skin samples were collected at zero, one, two, four, and six hours after the end of the exposure. Light microscopic evaluation was performed on formalin-fixed and paraffin-embedded skin sections. Hematoxylin and eosin stained sections were assessed and scored. A single topical exposure to meta-xylene, dimethamine, and sodium laurel sulfate resulted in granulocyte infiltration in rats as early as 2 hours. Segmental detachment of the epidermis from the dermal interface occurred in prolonged exposures of meta-xylene and dimethamine compared to the control groups.

These findings were interpreted to be the consequences of potential up-regulation of inflammatory mediators that result in skin damage. (Supported by CDC/NIOSH RO1 OH03654-03)

789 PYRIDOSTIGMINE BROMIDE SUPPRESSES IL-8 IN HUMAN EPIDERMAL KERATINO CYTES AND IN ISOLATED PERFUSED PORCINE SKIN EXPOSED TO DEET AND PERMETHRIN.

N. A. Monteiro-Riviere, A. O. Inman, R. E. Baynes and J. E. Riviere, Center for Cutaneous Toxicology and Restaure Pharmacology, North Carolina State University, Raleigh, NC.

Gulf War personnel were given pyridostigmine bromide (PB) as a prophylactic treatment against possible organophosphate exposure and then exposed to the insecticide permethrin (Pe) and the insect repellent DEET. Simultaneous exposure and the interaction of these compounds could contribute to the ill health effects in the Gulf War veterans. The purpose of this study was to assess the effects of DEET and Pb systemic PB on permethrin absorption. A large combination of topical mixtures of permethrin and/or DEET in various vehicles were applied to isolated perfused porcine skin flaps (IPPSFs). The absorption data has been reported elsewhere. Concentrations of interleukin-8 (IL-8) and TNF-α were assessed in the absorption studies to probe for potential inflammatory effects after complex mixture application. IPPSFs (n=4/treatment) were topically dosed with mixtures of Pe, DEET, Pb/DEET, and ethanol (EtOH). Each treatment was repeated with perfusate spiked with 50 ng/ml of PB. Treated IPPSs various effluent samples (0, 0.5, 1, 2, 4, and 8 hrs) were assessed by ELISA for IL-8 and TNF-α and by Elia for PGE2. Surprisingly, across most topical mixture combinations, release of IL-8 and TNF-α were significantly suppressed when Pb was infused into the IPPSFs. To probe the potential mechanism of this PB effect, human epidermal keratinocyte (HEK) cell cultures were exposed to Pe, DEET, Pb/DEET, with and without Pb in DMFSO vehicle. IL-8 was assayed at 1, 2, 4, 8, 12, and 24hrs. Pb suppressed IL-8 in permethrin treatments from 4 to 24 hrs supporting the IPPSF results. In conclusion, these studies clearly demonstrate that systemic exposure to PB suppressed the cytokine IL-8 release from keratinocytes. The relation of this finding to PB-induced changes in permeability absorption and/or other factors in the pathogenesis of Gulf War illness deserves further attention. (Supported by USAMRMC Grant DAMD17-99-C-9047)

790 LACK OF EFFECTS OF SULFUR MUSTARD AND JP-8 JET FUEL ON PERCUTANEOUS ABSORPTION OF SIMULTANEOUSLY ADMINISTERED TOPICAL PERMETHRIN AND DEET.

J. E. Riviere, J. D. Brooks, R. E. Baynes and N. A. Monteiro-Riviere, Center for Cutaneous Toxicology and Restaure Pharmacology, North Carolina State University, Raleigh, NC.

Systemic absorption of topically-applied permethrin and/or DEET have often been cited as potential toxins involved in the etiology of the Gulf War Illness. Previous studies in our laboratory have implicated pyridostigmine bromide (PB) as a potential modulating factor on permethrin dermal absorption. Others have postulated a toxicodynamic interaction with systematically absorbed permethrin and DEET. The purpose of the present study was to assess the effects of simultaneously administered JP-8 jet fuel or low-level (sub-blistering) sulfur mustard (HD) on permein
and DEET percutaneous absorption using the isolated perfused porcine skin flap (IPSF) model. IPSFs were topicaly dosed with 40 μg/cm² 1C-DEET and/or 75% DEET in various vehicholic vehicles with or without HD (40 μg/cm²) or 75% JP-8. A total of 40 IPSFs were studied (n=4 per treatment combination). Permeation absorption was measured by measuring radioactivity in perfusate and skin after completion of the perfusion experiments. DEET absorption was assessed by HPLC analysis of previous data, PB tended to enhance permeant absorption, detected as 1C-radioactivity. Co-administration of JP-8 slightly enhanced permeant absorption, however not to the extent that was seen with PB. DEET absorption was not affected. HD had no consistent effect on either permeant or DEET absorption or skin penetration. These results suggest that neither exposure to JP-8 for low level HD significantly increases percutaneous absorption of either topically applied permeant or DEET, and thus cannot be viewed as an additional risk factor for any role that these topically applied chemicals might play in Gulf War illness. (Supported by USAMRMC Grant DAMD-17-99-C-9907).

**ASSESSMENT OF CYTOTOXICITY TO HUMAN EPIDERMAL KERATINOCYTES AFTER EXPOSURE TO ALIPHATIC AND AROMATIC HYDROCARBONS.**

C. C. Chou, N. A. Miquel-Riurue, R. E. Bayles and J. E. Riurue, Center of Cutaneous Toxicology and Residue Pharmacology, North Carolina State University, Raleigh, NC.

Our laboratory has shown that jet fuel causes dermal toxicity and the release of the proinflammatory cytokine IL-8 from human epidermal keratinocytes (HEK). More than 200 aliphatic and aromatic hydrocarbons are commonly formulated in jet fuels but the cytotoxic effects of the individual hydrocarbons are unclear. One major problem being that vehicle effects often confound chemical toxicity studies. The purpose of this study was to assess the dermal toxicity induced by each jet fuel hydrocarbon using a single vehicle. Eight aliphatic (dodecane, undecane, tridecane and hexadecane) and aromatic (benzene, toluene, xylene and naphthalene) hydrocarbons at various concentrations in mineral oil were directly exposed on HEK. Cell viability at 24 hrs and IL-8 release from HEK at 4, 8, 12 and 24 hours were assessed. There was no significant decrease in cell viability by all hydrocarbons. However, there appears to be significant differences among each aliphatic and aromatic hydrocarbon with respect to their effects on IL-8 release. Not all HEK exposed to hydrocarbons at normal jet fuel concentrations of 0.5% to 5% increased IL-8 production. Exposure to mineral oil alone for 30 min did not affect cell viability or IL-8 release. In conclusion, we have demonstrated that mineral oil is a suitable vehicle for studies in HEK culture systems especially for high-dose, short-term exposures of compounds with a wide range of lipophilicity. Utilizing 96-well plates also allows for the evaluation of cytotoxic effects of individual and clusters of hydrocarbons to be screened in a relatively short period of time. (US Air Force Office of Scientific Research F49620-01-1-0080).

**DERMAL ABSORPTION OF AVERMECTIN: FORMULATION AND SPECIES DIFFERENCES.**

B. Barlow and R. E. Bayles, Center of Cutaneous Toxicology and Residue Pharmacology, North Carolina State University, Raleigh, NC.

Avermectin are approved for topical application in cattle only. There is however some concern that topical application in other domestic animals, may result in significant dermal absorption and possible cutaneous side effects in animal-derived food products. The primary aim of this study was to assess dermal disposition of [1H]avermectin in skin from food-producing species in vitro using commercial alcohol-based and oil-based formulations. Skin samples from cattle, sheep, goats, and pigs were perfused in a flow-through diffusion cell system for 8 hours. Skin sections were perfused at 150 μg/cm² of [1H]avermectin in 20 μl of 75% isopropanol:25% cromolin. 100% mineral oil, or 100% isopropyl alcohol. Perfusion samples were collected at various time points and surface swabs and dose skin samples were obtained at 8 hours. [1H]avermectin absorption ranged from 0.09% - 0.20% dose and there were no significant differences between formulations for [1H]avermectin absorption in each species. The presence of isopropyl alcohol significantly increased skin deposition in all species when compared to the oil formulation. Absorption was significantly greater in cattle skin than in pig skin for the isopropanol alcohol formulations, but there were no significant species differences for the hydrocarbon formulation. While all 11.49 - 53.2% dose retention. These results suggest that the alcohol-based formulations enhanced H-avermectin absorption and skin deposition in some animal species, and that this effect is more likely to be observed in ruminant species than in porcine species. (Supported by North Carolina State University Grant FR&P).

**CUTTING FLUID FORMULATIONS INFLUENCE THE DERMAL DISPOSITION OF LINEAR ALKYLBENZENE SULFONATE (LAS).**

R. E. Bayles, J. D. Books, B. Barlow and J. E. Riurue, Center of Cutaneous Toxicology and Residue Pharmacology, North Carolina State University, Raleigh, NC.

Linear alkylbenzene sulfonate (LAS) is often added as a surfactant to cutting fluid formulations to enhance the performance of metal machining operations. Unfortunately, LAS and other cutting fluid additives are in workers' hands in the machine tool industry. The purpose of this study was to assess skin permeation and deposition of [1C]-LAS when topically applied to intact membranes (porcine membranes) and skin in vivo through-flow diffusion cell system as mineral oil or polyethylene glycol (PEG) mixtures. [1C]-LAS mixtures were formulated with 3 or 5% isopropanol, 0% or 2% cromolin (TRI), 0% or 5% Triton X-100 (TRE), and 0% or 5% sulfated sodium palm kernel (SAK) as follows: TRI, TEA, SRA, TRI+TEA, SRA+TEA, SRA+TEA+TRI, TEA+TRI+SA, SRA+TRI+SA, SRA+TRI+SA+TEA+TRI. In silico membranes, LAS absorption ranged from 0.09% - 0.18% dose, and there were no differences between corresponding mineral oil and PEG mixtures. Membrane levels were greater with TRI only in mineral oil and PEG mixtures. In porcine skin, [1C]-LAS absorption ranged from 0.6% - 0.32% dose, and there were significant differences between several mineral oil and PEG mixtures. LAS penetration into stratum corneum (SC) was often greater in mineral oil than in PEG mixtures. Surprisingly, LAS absorption was significantly greater in pig skin than in silastic membranes for PEG mixtures containing TRI+TEA. These observations suggest that although very little LAS is absorbed, cutting fluid components can alter LAS deposition into the SC and skin. Furthermore, chemical-biological interactions in viable skin with synegism with a biocide (TRI) and an amine (TEA) may be important determinants for LAS disposition in skin. (Supported by NIOSH Grant R01-OH-03669).

**BIOMECHANICAL MONITORING TECHNIQUES TO ASSESS SULFUR MUSTARD LESIONS IN WEANLING PIGS.**

F. M. Reid, J. D. Waugh, N. A. Nimeth and J. S. Graham, Medical Research & Evaluation Facility, Battelle Memorial Institute, Columbus, OH and U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. Sponser: C. Olson.

The chemical warfare agent, sulfur mustard (SM) produces severe skin injury and there is no established medical treatment as objective, quantitative, and non-invasive means for conducting SM burn assessments to evaluate medical treatments. Additionally, assessments were confirmed by histopathological evaluation. Six animals per group were exposed to SM (2 or 30 min exposure to 400 μl SM applied to each of 6 abdominal sites) and six animals received sham control (400 μl deionized water for 30 min) exposure. Each site was evaluated on days 0 and 2. Two- and 30 min groups were significantly different from control and significantly different from each other, as characterized by histopathological endpoints of burn depth, basal cell necrosis, depth of necrosis, and vascular necrosis. Twenty-two and 30 min dermal burns were significantly different from control using redness (Chroma Meter) and transcutaneous water loss (Evaporimeter), but not significantly different from each other. The 2 min burns and control were significantly different from 30 min burns for skin thickness (Ultrasound). Compared to control, the 2 min burns were significantly increased and the 30 min burns were somewhat decreased for microradiography blood flow (Laser Doppler). We demonstrate that both biomechanical and histopathological evaluations are useful methods for characterizing SM burns in a weanling pig model. (Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-C-9905).

**α-AMYL CINNAMALDEHYDE (α-ACA) AND α-HEXYLCINNAMALDEHYDE (α-HCA) DO NOT PRODUCE DERMAL SENSITIZATION OR CROSS-SENSITIZATION IN HUMANS.**

C. S. Letizia and A. M. Api, Research Institute for Fragrance Materials, Inc., Hackensack, NJ.

Both α-ACA (heptanal), 2-(phenylmethylene)- and α-HCA (cinnamal, 2-(phenylmethylene)-) are important fragrance ingredients. Studies were conducted to evaluate the potential of these two materials to induce sensitization and cross-sensitization in a normal human population. Sensitization was evaluated using either a modified Draize human repeated insult patch test (HRIPPT) procedure consisting of nine 24 hour occluded induction applications followed two weeks later by a 24 hour occluded challenge application; or by a human challenge test procedure consisting of 8 occluded induction applications to the same site for five alternate-day 48 hour periods followed ten to fourteen days later with a 48 hour occluded challenge application. Cross-sensitization between these two materials was also evaluated using a HRIPPT procedure. α-ACA produced no sensitization in 71 human
volunteers when tested at 6% in human maximization tests or in 95 human volunteers when tested at 20% in a HRIPT. α-HCA produced no sensitization in 81 human volunteers when tested at 12% in human maximization tests or in 234 human volunteers when tested in HRIPT at dose levels ranging from 5% to 20%. Cross-sensitization was not observed in 95 subjects tested with 20% α-ACA and then cross-challenged with 20% α-HCA or in 95 subjects tested with 20% α-HCA and then cross-challenged with 20% α-ACA. Although sensitization has been reported in animal tests, the results of these studies indicate no evidence of delayed sensitization with α-ACA or α-HCA. Additional studies were performed with trichloroethylene.

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A NOVEL TECHNIQUE TO STUDY PERCUTANEOUS ABSORPTION BY USING A SILICATIC MEMBRANE COATED FIBER.
Center for the Study of Toxicology and Residue Pharmacology, North Carolina State University, Raleigh, NC; and Protec-Tex Residue Research Laboratory, Department of Toxicology, North Carolina State University, Raleigh, NC.

Silicate membrane coated on a section of fused silica fiber has been used successfully as an analytical tool to extract analytes from aqueous solutions. In this study, the silicate coated fiber was used as a permeation membrane to study the percutaneous absorption of model pesticides. It represents a half compartment of the conventional Franz diffusion cell, which allows for more detailed permeation kinetics to be investigated. The membrane coated fiber was immersed in the magnetic stirred donor phase to-accelerate the diffusion of the solution, and the fiber was then transferred directly into the injection port of a Gas Chromatograph/Mass Spectrometer for quantitative analysis. This technique can assess the permeation amounts at various permeation times, stirring speeds and donor compositions. A theoretical model was proposed to describe the permeation processes of the silicate membrane coated fiber in terms of diffusion parameters. This model allows for the boundary layer between the donor phase and the silicate membrane to be considered; its thickness is assumed to be constant under steady state diffusion. The permeation kinetics of 30 different compounds having a wide range of partition coefficients were examined utilizing silicate membrane coated fiber. The experimental permeation flux and time profiles were well described by the proposed mathematical model. The thickness of the boundary layer, the diffusion coefficients in the donor phase and in the silicate membrane, and the partition coefficients can be measured simultaneously with this novel technique. This approach would foster development of high throughput determination of skin diffusion parameters. (Supported by NIOSH R01 OH 43660 and 07553)

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A GENOMICS APPROACH FOR THE DEVELOPMENT OF AN IN VITRO METHOD FOR SKIN SENSITIZATION.
The Procter & Gamble Company, Cincinnati, OH and Syngenta CTL, Macclesfield, United Kingdom.

For the development of an in vitro method for predictive skin sensitization testing, a genomics approach is being used to elucidate pathways that are involved in the immune recognition of chemical allergens by dendritic cells (DC) and T-cells, the key cells involved in the induction and elicitation of allergic contact dermatitis. The overall aim is to identify and characterize those genes in both DC and T-cells which provide sensitive, selective and robust markers of skin sensitization and which allow discrimination between contact allergens and skin irritants. To date, there are no published reports describing genomic-scale analysis of the early changes induced in either T cells or DC resulting from antigen-specific interactions between the self cells and foreign antigens. The limited number of reports regarding microarray analysis of T cell activation following in vitro re-stimulation of T cells with live antigen revealed no detectable difference in gene expression. The current project is investigating the in vitro gene expression changes in gene expression that are induced by antigen-specific T-cells/DC, using high throughput expression analysis. Gene expression analysis was conducted using the Affymetrix GeneChip® human U95Av2 array. Changes in gene expression of 2-fold or more were observed in 108 genes, ranging from an up-regulation of 42-fold to down-regulation of 10-fold. Information gained in this study will be used as a benchmark in the examination of primary responses in non-sensitized T cells co-cultured with hapten-treated DC.

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INHIBITION OF PHOTOCARCINOGENESIS BY TOPICAL ADMINISTRATION OF DAPSONE.
The Center for Photobiology, Argus Research, Hormah, PA and Arix Laboratories, Fort Collins, CO.

Dapsonate (DAP) is a potent anti-inflammatory and anti-bacterial approved as an oral therapy for leprosy and dermatitis herpetiformis. While effective for these diseases and acne, the relatively high oral doses of DAP can cause adverse events. Topical administration would permit the use of lower DAP concentrations to skin but greatly reduce the systemic dose and side effects. Because of the high absorbance of DAP in the ultraviolet portion of the solar spectrum and its anticipated chronic topical administration to facial skin exposed to sunlight, a photocarcinogenesis test assessed the potential of topical DAP to modify the development of solar-simulated radiation (SSR)-induced skin tumors in C3H/Ka:HR hairless mice. Dapsonate was formulated in a dichloro glycol monoester (DGMES)-based vehicle at DAP/DGMES ratios of 1%/10%, 3%/17.5% or 5%/25%. The study design included a group of mice administered vehicle (25% DGMES) and low and high UVB calibration groups. Mice were administered formulations and exposed to SSR five days per week for an additional 12 weeks. Clinical observations and tumor mapping were performed weekly. Cumulative responses indicated DAP administration reduced the incidence of erythema, edema and thickening in the groups of mice administered the DAP/DGMES formulations as compared with mice exposed to SSR alone. Dapsonate concentration-dependent hyperactivity occurred throughout the 40 week dosing period. The incubation median latens weeks to tumor, sexes combined, were 41.50, 47.50, 48.00 and 49.90 weeks for the vehicle, low, middle and high dose DAP/DGMES formulations, respectively, compared to 41.50 weeks for the low UVB calibration group, indicating a delay in tumor development. DAP/DGMES formulation-treated groups and no vehicle effect. In conclusion, topical DAP administration does not enhance photocarcinogenesis in the hairless mouse model and indeed affords protection against SSR-induced skin tumor development and cumulative responses to SSR exposure.

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ABSORPTION OF 14C-CYCLOTRIMETHYLHYDROXYTRIMETHYLLAMINE (RDX) THROUGH HUMAN SKIN IN VITRO.

Dermal exposure of the explosive chemical RDX can occur to humans during the manufacturing process and occupational (load, assemble and package) operations. There is no information on dermal absorption of RDX in humans for risk assessment. We studied dermal absorption of RDX in human skin in vitro in flow-through diffusion cells. RDX (38.4 ppm or 0.5 ppm in acetone 100 μL) was applied to the skin and collected as diffused receptor fluid for every 6 h up to 24 h. At the end of the experiment, the unabsorbed RDX was washed with soap water and water with cotton swabs, and radioactivity present in washings was determined. The RDX absorbed or penetrated in the skin was also determined by separating stratum corneum, epidermis and dermis at the end of the experiment. Our results show that the total of approximately 6% of applied dose was absorbed in the skin (receptor fluid and skin) in 24 h. The absorption of RDX in the receptor fluid was relatively slow with a little 1.28% of applied dose in 24 h. Preliminary results of analysis of stratum corneum (tape strip), epidermis and dermis revealed that a majority of radioactivity localized in the upper stratum corneum and epidermis and it was very low in the dermis of the skin after 24 h. The total recovery of applied dose (receptor fluid, skin and washings) was about 80%. These results show that RDX is absorbed in the skin but diffusion through skin was relatively slow. This may be due to liposolubility of RDX in the skin. The estimated level of RDX in the skin can be used to evaluate health risks associated with dermal exposure (Abstract does not reflect US Army policy).

800
IN VITRO HUMAN SKIN PENETRATION OF THE RADIOLABELLED FRAGRANCE MATERIALS, AHTN AND HHCB.
in vivo human skin permeation rate and distribution of two radiolabelled polycyclic musk, AHTN and HHCB, following applications under normal conditions was determined. The studies followed the European Scientific Committee on Cosmetic Products and Non- Food Products (SCCNFP) guidelines and used human cosmetic reduction skin from surgery. Screening studies were conducted to identify the most appropriate receptor fluid, which for these studies was 6% of the surfactant Volpro20 in physiologically balanced saline. The skin samples were heat separated and the epidermal membranes comprising both the stratum corneum and the epidermis were used. Because the system was static, the dermis was stripped
away. The integrity of the membranes was determined prior to dosing using tritiated water. A 1% solution of radiolabeled (14C) test material in ethanol was applied to the membrane (20 pl/sq cm). After 24 hours, the amount of material in the receptor fluid was determined using a scintillation counter. Mass balance was determined and included test material collected from the surface wash, 10 skin stripings, remaining epidermis, receptor phase, donor chamber, and filter paper supports. After 24 hours, 38% of the added AHTN dose remained on the AHTN membrane. The total absorbed dose for AHTN, determined by adding the amounts of test material in the remaining epidermis, the receptor phase and the filter paper was 4.1% of the applied dose. The evaporative loss was 2.9%. Overall recovery of applied AHTN was high at 92.5% or 93.5% when loss due to evaporation was included. For HHCb, the penetration through th e dermal membranes into the receptor phase. The total absorbed dose for AHTN, determined by adding the amounts of test material in the remaining epidermis, the receptor phase and the filter paper was 5.2% of the applied dose. The evaporative loss was 2.4%. Overall recovery of applied HHCb was good at 92.1% or 94.5% with evaporative loss included. The results of these studies show that the permeant absorption of both of these fragrance materials was low - 4.1% for AHTN and 5.2% for HHCb.

801 IN VITRO HUMAN SKIN PENETRATION OF SEVEN RADIOLABELLED FRAGRANCE MATERIALS.

in vivo human skin penetration studies on 7 fragrance materials were conducted according to the FDA guidelines. The materials studied were acetyl cedrene, estragole, methyl 2, 4, 3, 3, 5, 5, 6, 8-octadec-2-yl, 3, 4, 7-ethylenediamine hydroxy ethanol (MMDHCA) and 1, 2, 3, 4, 5, 1, 2-ethanethiol (OTNE). Each of the test materials was radiolabeled with 14C. The full thickness human skin samples were either from the breast or abdomen. The skin samples were heated and the epidermal membranes were mounted on filter paper supports. The supports, with the membranes, were then placed onto diffusion cells and trimmed to size. The integrites of the membranes were determined using tritiated water. Twenty pl of a 1% solution of each material in ethanol was applied to the surface of the membranes. At 8, 24, 36, and 48 hours 200 pl samples of the receptor fluid (50% ethanol/water solution) were taken from the receptor chamber and analyzed by liquid scintillation chromatography. Evaporative loss of each fragrance material was also determined.

At 24 and 48 hours the following percentages of the applied dose permeated into the receptor phase: 6% and 11% of acetyl cedrene, 17% and 10% of estragole, 14% and 20% of methyl arsine, 31% and 40% of methyl 2, 4, 3, 3, 5, 5, 6, 8-octadec-2-yl, 3, 4, 7-ethylenediamine hydroxy ethanol and 36 and 34% of methyl eugenol, 42 and 50% of MMDHCA, and 9 and 15% of OTNE. These results suggest varying permeant absorption of these materials. However, it should be noted that the donor phase became somewhat depleted after evaporation and could affect the amount of test material available for absorption.

802 INVESTIGATION OF THE SENSITIZATION POTENTIAL OF MUST KETONE USING THE GUINEA PIG MAXIMIZATION TEST (GPMT).

As part of a risk assessment on musk ketone, a widely used synthetic fragrance ingredient, a Guinea Pig Maximization Test (GPMT) was used to investigate the sensitization potential of this material. The GPMT was conducted according to OECD Guidelines 406, and Good Laboratory Practices were followed. Preliminary testing determined dose levels for the main study. Exposure during the induction phase of the GPMT consisted of a pair of intradermal injections with 5% musk ketone followed by a 48-hour occlusive dermal application with 75% musk ketone seven days later. After a 2-week rest period, the challenge phase consisted of 24-hour occluded dermal applications with three concentrations: 75, 25, and 7.5% musk ketone. The vehicle used for all phases was 9:1 olive oil/acetone. Skin sites were examined at 24 and 48 hours following patch removal, erythematous reactions were quantified and scored. No irritation responses were observed, and the test was maximized. At 75% /20 test animals and 1/10 control animals responded; and at both 25 and 7.5% /20 test animals and 0/10 control animals responded. According to OECD guidelines, this material is not considered a sensitizer requiring a label, since there was less than a 30% response rate.

803 INHIBITION OF FLK-1/KDR, PDGFR and FGFR DOES NOT ALTER HEALING OF INCISIONAL SUTURED SKIN WOUNDS IN MICE.

The control of angiogenesis during tissue repair events is crucial importance and disturbances of these processes have been suggested to play a role in impaired skin wound healing. Vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are the major angiogenesis factors, although other growth factors can also modulate angiogenesis during wound repair. Using an incisional wound model in SKH1 hairless mice, we evaluated the effects of therapeutic and supratherapeutic doses of SU540668, an inhibitor of PDGFR (Ki = 0.08 µM), Flk-1/ KDR (Ki = 2 µM) and FGFR (Ki = 1.2 µM), on the healing of skin wounds. Mice were administered vehicle, SU540668 (100 µg/kg/day and 400 µg/kg/day, i.d.), or dexamethamine (1 mg/kg/day; positive control) continuously for 6 days prior to wounding and for 28 days post-wounding. A full-thickness incision was made on the dorsum of mice and closed with three equidistant sutures. Wound healing was monitored histologically and using a tensiometer up to 4 weeks post-wounding. SU540668 at therapeutic doses (100 µg/kg) had no significant effect on the tensile strength and macroscopic and microscopic morphology of the wounds, whereas dexamethasone treatment resulted in skin apero, decreased tensile strength, and delayed epidermal and dermal restoration. At supratherapeutic doses (400 µg/kg), where SU540668 may not specifically inhibit other receptor tyrosine kinases, there were subtle transient histomorphologic changes and slight decreases in tensile strength of minimal biological significance, suggesting a slight delay in the wound healing process up to 14 days post-wounding with complete return to control levels thereafter. In conclusion, these data indicate that PDGFR, Flk-1/ KDR and FGFR inhibition at levels necessary to inhibit tumor growth in mouse xenograft models does not affect the healing of incisional wounds and that redundant pathways likely compensate for inhibition of PDGF, VEGF and FGF function in normal cellular processes.

804 EFFICACY OF DIPHOTERINE HCl. DECOMPOSITION IN RATS: A COMPARATIVE STUDY.
A. H. Hall, M. Cavallini, M. M. Corsi, L. Marzio, L. Blomer, and J. Blomer, Department of Emergency Medicine, Division of Toxicology, El Paso, TX.

Efficacy of Diphotterine (R) HCl Decomposition in Rats. A comparative study. Cavallini, M. Corsi MM, Marzio L. Blomer, J. Hall AH. Twenty-five male rats had dermal exposure to 25% HCl for 15 seconds. Five control rats had no decomposition. All applicable animal use guidelines were followed. The other groups of 5 rats each were decommissioned as follows: saline solution; calcium gluconate; Diphotterine. Assays of IL-6, TNF-alpha, NO, and substance P indicated that Diphotterine was more efficacious than the other decomposition solutions.

805 CALIBRATION OF IN VITRO DERMAL ABSORPTION TEST SYSTEMS: INTER-SYSTEM VARIATION IN TRITIATED WATER KP VALUES.

The skin permeability coefficient (KP) is used to predict dermal systemic exposure to environmental contaminants. Since many different in vitro dermal absorption test systems have been reported in the literature for measuring KP values, our laboratory conducted inter-system tests for calibration purposes. Tritiated water (H2O) was selected as the test chemical since the Kp of H2O is often used to test human skin barrier integrity. Our tests were conducted following standard ISO test guidelines. Three different flow-through, in vitro systems (Testa), three different flow-through systems (Bobraugh), Amie and AIDA systems. The Braough and Amie systems were obtained commercially, the Automated In vitro Dermal Absorption (AIDA) system was developed in-house. All three systems used flow-through donor and receiver solutions. For the Braough system which used a non-flowing static donor reservoir, the Braough, Amie and AIDA systems obtained average (H2O) KP values (units of cm X 109/hr) of 2.3 + 1.38 (n = 6), 1.0 + 0.40 (n = 6), and 0.5 + 0.11 (n = 4), respectively. Normalized for the Braough Kp data, the average KP was 2.4 and 4.5 fold lower for the Amie and AIDA systems, respectively. As test were conducted with fresh/unfrozen human breast skin from the same donor and all environmental conditions (e.g. temperature, donor and receiver solution concentrations/compartment) were kept the same, the reason for an intra-system difference in KP values was not apparent. The higher KP obtained for the Braough system was not considered to be due to is use of a static donor supply since a flowing donor should act to stir the donor solution, and if anything increase the absorption rate. Given the range in standard deviation in the average Kp data reported here, further comparative tests using H2O and other chemicals to examine the need for developing inter-system calibration factors.

806 DIETHANOLAMINE (DEA). IN VITRO DERMAL ABSORPTION IN FUZZY RAY SKIN.
S. J. Kounek, A. Marks, R. J. Lachheb and P. L. Bronaugh. Office of Cosmetic and Colors, USDA, Laurel, MD and Department of Polymer Science, University of Southern Mississippi, Hattiesburg, MS.

Diethanolamine (DEA) is used in cosmetic formulations as an emulsifier, thickener, wetting agent, detergent, and foam booster. DEA is reported to be used in mainly wash-off cosmetic formulations. DEA is also used in the preparation of
DEA conjugates are used most often in wash-off products, but they are also used in leave-on products. Unreacted DEA concentrations in DEA condensate cosmetic raw materials may be as high as 20%. Concerns about DEA's safety have been raised by the National Toxicology Program due to the carcinogenicity testing in rodents. Therefore, we initiated in vitro studies to measure DEA's acute rat dermal absorption. [14C]DEA (approx. 0.1 μCi/cell) skin absorption was measured by using one dosing vehicle which was a spiked consumer lotion product applied at 3.0 mg/cm². The dosing vehicle containing DEA was applied to skin in the diffusion cells for 24 hr, then the skin was washed. Absorption was measured over a period of 72 hr by using flow-through diffusion cells (0.64 cm²/cm) with a receptor fluid consisting of HBBSS (pH 7.4). In rats (n=3), the percentage of applied dose absorbed over 24 hr was 1.4 ± 0.5 (mean ± SEM) with approximately 4% remaining in skin. At 72 hr after DEA application, the percentage of applied dose absorbed was 1.5 ± 0.5 with approximately 4% remaining in skin. At 24 hr, there was 1.0 ± 0.9 and 1.9 ± 0.5 of the applied dose remaining in the stratum corneum and epidermis/dermis, respectively. Skin levels of DEA did not change even when followed for 72 hr. These studies indicate that little of the DEA that was found in the skin at 24 hr diffused through the skin to be absorbed into the receptor fluid. Therefore, skin levels of DEA should not be included in the estimate of systemic absorption used in an exposure assessment.

8098 IN VITRO PERCUTANEOUS ABSORPTION OF DIETHANOLAMINE (DEA) IN HUMAN SKIN.


Diethanolamine (DEA) is an amine alkylamine contained in pharmaceuticals and various cosmetic and personal care products, either as a conjugate (DEA condensates and DEA salts) or as a constituent of triethanolamine (TEA). Concerns about the safety of DEA have been raised by the National Toxicology Program (NTP). Therefore, we initiated studies to measure the extent of DEA absorption in human skin relevant to exposures from consumer products. DEA penetration was determined from three different product classes: shampoos, hair dyes and body lotions.

Two commercial products from each class were spiked with [14C]DEA and applied to excised viable and non-viable human skin mounted in flow-through diffusion cells maintained with a physiological buffer (HBBSS, pH 7.4) as receptor fluid. Products remained on the skin for 5 min, 30 min and 24 hr for shampoos, hair dyes and body lotions, respectively. At the end of 24 hr, the amount of DEA absorbed in skin layers and the receptor fluid was determined and expressed as the percent of the applied dose absorbed for each product type. Most of the absorbed dose was found in skin: 2.88%, 2.98% and 10.0% for shampoos, hair dyes and body lotions respectively. Only small amounts penetrated into the receptor fluid: 0.088%, 0.096% and 0.09% for shampoos, hair dyes and body lotions respectively. In skin absorption studies extended to 72 hours only small amounts of additional DEA were absorbed into the receptor fluid. Therefore, skin levels of DEA should not be included in estimates of systemic absorption used in exposure assessments.

8100 RISK ASSESSMENT OF ORGANOPHOSPHORUS CHEMICALS INCLUDING CHEMICAL WARFARE AGENTS.


1Dermatology, University of California, San Francisco, CA and 2Edgewood CB Center, Aberdeen Proving Grounds, MD.

The objective was to perform analysis on existing dermal absorption data on selected organophosphorus compounds including chemical warfare agents - sarin (GB), soman (GD) and VX - and develop a postulate of critical factors in observed regional differences in toxicant uptake/absorption by human skin. Body contours for skin absorption and lethality were developed. Parathion and malathion exposed head and neck region (s4), trunk (s3) and genital area (s12) more chemical than arms and hands, and legs and feet. In field use, parathion has caused human death while malathion is considered safe. Permeability constants (Kp) (potential chemical absorbed through human skin per unit area and time) indexed to regional variation gave the most chemical absorbed through all regions of the human body. Human skin is more permeable to parathion than malathion. Further overlap of toxicity data to the construct showed lethality with parathion but not for malathion. VX has a high Kp and is also the most toxic; 50% lethality is reached not only for total systemic exposure but also for just trunk exposure in the first hour. In later hours, 50% lethality is reached when exposure is to any single region of the body. Sarin and soman are less toxic percutaneously than VX, and they have the same toxicity level. However, soman has greater skin absorption than sarin. Estimates of 50% lethality are only reached for sarin at the 24-hour exposure level, whereas the 50% lethality estimate for soman is reached in the first hour. Chemical warfare agents are a serious threat to human life. In order to minimize or prevent the hazard of these agents, we need to understand the way that these chemicals get on and into the human body, both for military function and protection of life.

8078 EVALUATION OF AN IN VIVO MODEL: HUMAN SKIN GRAFTED ON "NUDE" MICE TO PREDICT DERMAL ABSORPTION IN HUMAN.

A. P. Luykx1, S. Diers1, J. P. Marty1 and N. Carmichael, 1Experimental Toxicology, Avenir Consult, Sophia-Antipolis, France and 2Laboratoire de Dermopharmacologie, Faculté de Pharmacie, Chatenay-Malabry, France.

Dermal absorption data from an in vivo model based on human skin grafted on "nude" mice were compared with data from human volunteer studies and two other models currently used (rat in vivo model and in vitro human skin model using diffusion cell) for three lipophilic chemicals. Results indicate that for linoleate and cypermethrin dermal penetration and diffusion were similar for the human skin grafted on "nude" mice and diffusion cell models. For malathion, although similar diffusion was found in in vitro and grafted human skin, the amount remaining in stratum corneum (SC) was three times higher with diffusion cell model. Rat skin is more permeable than human skin independently of the human skin model used. Absorption through human skin in diffusion cell and human skin grafted on mice were two times higher than absorption in human volunteers. Inclusion of SC content in total absorbed dose would markedly overestimate absorption in human volunteers for malathion and cypermethrin. So, the fate of the high amount of cypermethrin in SC has been evaluated both with human skin grafted on mice and the rat in vivo. Four days after decontamination, 15.4% of cypermethrin was still present in SC of rat skin compared to only 2.5% in human skin grafted on "nude" mice. Further, results with human skin grafted on "nude" mice indicate that only one-third of the amount of cypermethrin remaining in SC at the end of exposure period was absorbed while the remainder was presumably lost through desquamation. This work indicates that the diffusion cell using human skin is a good model for assessing absorption of chemicals. However for a lipophilic compound stored in stratum corneum, an in vivo model like the human skin grafted on "nude" mice may predict more accurately the level of absorption in human.

8102 ALLERGEN-INDUCED CHANGES IN INTRACELLULAR EPIDERMAL TUMOR NECROSIS FACTOR α EXPRESSION.

S. Campos, M. Cumberbatch, R. J. Dearman and L. Kimber, Immunogenetics Central Toxicology Laboratory, Macclesfield, United Kingdom.

Following topical exposure of mice to skin sensitizing chemicals, mRNA expression for a number of epithelial cytokines is upregulated, including that for tumor necrosis factor α (TNF-α). To measure directly intracellular TNF-α protein at the single cell level, a two-colour flow cytometric method for the detection of cytokine-producing keratinocytes and Langerhans cells (LC) prepared ex vivo from BALB/c strain mice has been developed. We report that the process of trypanerpitation of dorsal ear halves to prepare epidermal cell suspensions results in the production of TNF-α by the majority of keratinocytes, with the subsequent induction of other epithelial cytokines. However, the induction of Langerhans during epidermal cell preparation and fixation early in the isolation procedure with formaldehyde reduced significantly non-specific upregulation of TNF-α expression. Using this approach, permeabilization of epidermal cell suspensions with saponin and two-colour staining for a number of cytokines together with MHC class II revealed approximately 3-5% of keratinocytes derived from naive mouse skin to be positive for TNF-α. Under these conditions it was also found that a proportion of keratinocytes (approximately 20%) was associated with interleukin (IL)-6 and IL-12 expression, with approximately 40% exhibiting IL-10. At least 70% of LC were positive for intracellular IL-6 and IL-10, with only a minority population of LC displaying intracellular IL-12. Following topical application on the dorsum of both ears with the contact allergen oxazolone (Ox: 0.5%), enhanced intracellular TNF-α expression by a small proportion of keratinocytes, relative to vehicle treated mice, was observed within 30 minutes of exposure. Further increases of up to 10% in the frequency of keratinocytes positive for TNF-α were detected 1-4 hours following exposure to allergen. Taken together these data suggest that this technique provides a useful approach to the investigation of changes induced in epidermal cytokine expression as a result of skin sensitization.
811 EVALUATION OF THE DERMAL ABSORPTION OF METHYL ETHYL KETONE IN F344 RATS USING REAL-TIME BREATH ANALYSIS AND PBPK MODELING.
A. D. Woodcock and K. D. Thrall. Molecular Bioscience, Battelle, Pacific Northwest Division, Richland, WA.

Methyl ethyl ketone (MEK), a high boiling solvent, is used as a component of a variety of paints and other solvents. Dermal exposure to MEK can result from the occupational or consumer use of paints and other commercial products. To understand the significance of these exposures, the dermal bioavailability of MEK was assessed in F344 male rats using a combination of real-time exhaled breath analysis and a physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to MEK at 5 mg/ml aqueous concentration using a 2.5 cm diameter occluded glass pach system attached to a clipper-shaved area on the back of the rat. Immediately following exposure the animal was placed in a glass off-gassing chamber and exhaled breath was monitored as chamber concentration in real time using an ion trap mass spectrometer (MS/MS). This real-time methodology allowed to discern the uptake, peak concentration and clearance phases associated with the dermal exposure. For example, the exhaled breath profile clearly demonstrated the rapid absorption of MEK, with peak chamber concentrations ranging from 20 to 25 ppm observed within 30-60 minutes from the start of exposure. The PBPK model describes the exposure and off-gassing system was used to estimate a single dermal permeability coefficient to describe all of the exhaled breath data for n=6 animals. These rat studies using aqueous MEK will form the basis for comparing the absorption of MEK in various paint products and may ultimately aid in understanding human health risk under a variety of exposure scenarios. (Supported by NIOSH 1-R01-OH03658-01A2).

812 ASSESSMENT OF DERMAL IRRITATION OF THREE BENZENE SULFONATE COMPOUNDS.

Three benzene sulfonate compounds are present in groundwater near a former disposal site at concentrations ranging from < 1 mg/l to < 500 mg/l. Dermal irritation studies were performed for each of the three sulfonate compounds to determine if they would cause irritation to the skin of persons using water containing the compounds for bathing, showering, or other uses where skin would be exposed. The three compounds are: (1) benzene sulfonate, (2) benzene meta-sulfonate, and (3) para-hydroxybenzene sulfonate. The studies were performed in accordance with USEPA’s Health Effects Test Guidelines: OPPTS 870.2500, Acute Dermal Irritation, 1998. At the highest dose tested (5,000 mg/l), all three sulfonate compounds were considered to be irritating. In all cases, the reactions were reversible. At the second highest dose tested (2,000 mg/l), benzene meta-sulfonate was not considered an irritant while para-hydroxybenzene sulfonate was considered a slight irritant, producing very slight to mild erythema in all test animals. At the lowest dose tested (1,000 mg/l), benzene sulfonate was not considered an irritant at 1,000 mg/l or at 500 mg/l. It is important to note that all three sulfonate compounds produced only a slight irritation at the highest dose tested. No compound produced edema or a severe irritation (i.e., severe erythema). Furthermore, all irritation responses at the highest dose tested were reversible within 72 hours, and the only irritation response observed at the second highest dose (2000 mg/l of benzene sulfonate) was reversible in less than 24 hours.

813 ASSESSMENT OF DERMAL PENETRATION OF THREE BENZENE SULFONATE COMPOUNDS.

Three benzene sulfonate compounds are present in groundwater near a former disposal site at concentrations ranging from < 1 mg/l to < 500 mg/l. Dermal penetration studies were performed for each of the three sulfonate compounds to determine if they would be systemically absorbed by persons using water containing the compounds for bathing, showering, or other uses where skin would be exposed. The three compounds are: (1) benzene sulfonate (BSA), (2) benzene meta-sulfonate (BMS), and (3) para-hydroxybenzene sulfonate (PHBS). The dermal penetration studies were performed with infinite doses of 2,000 mg/l aqueous solutions of these compounds in vitro for 24 hours with human abdominal skin using Franz diffusion cells. The skin samples were obtained from up to a minimum of three donors. The integrity of human skin was tested with 31-H2O before use. Aliquots of receptor fluid were collected over time intervals and used to determine liquid chromatography (HPLC) and ultraviolet absorption. HPLC conditions were 534/270, 25 mm tetrahydrofuran/methanol/methanol/isotonic. Analytical wavelengths were 263, 267 and 271 nanometers for BSA, BDSA and HBSA, respectively. No sulfonate compounds were detected in the receptor fluid at 24 hours for any of the test compounds. The results of this study confirm the results of a pilot study performed using the same methods but different chromographic conditions.

814 MECHANISMS OF UVB LIGHT-INDUCED SUPPRESSION OF NITRIC OXIDE PRODUCTION IN MURINE KERATINOCYTES.
R. Sur, T. M. Mariano, D. E. Heck and J. D. Laskin. 1Environmental and Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ and 2Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.

Ultraviolet light of high energy and shorter wavelengths (UVB, 290-320 nm) is utilized therapeutically to reduce irritation in inflammatory dermatoses. The mechanisms underlying the actions of UVB are unknown. We hypothesize that the UVB-induced reduction in nitric oxide production may be due to the inhibition of inducible nitric oxide synthase (iNOS) in response to the inflammatory cytokine, gamma interferon. In the present study we examined the effects of UVB light on the activity of NOS2 in keratinocytes. We found that UVB light suppressed gamma-interferon-induced nitric oxide production. We also found UVB light was dose dependent in the range of 2.5-25 mJ/cm2. In this range, UVB light was found to inhibit the synthesis of NOS2 mRNA and protein expression in keratinocytes, as determined by RT-PCR and western blotting, respectively. We found that two transcription factors known to be important in regulating expression of iNOS are significantly reduced by UVB light. Taken together, our data suggest that UVB light functions to suppress expression of NOS2 by inhibiting activation of key transcription factors important in regulating expression of iNOS. Our findings support the hypothesis that UVB light inhibits NOS2 gene expression in keratinocytes.

815 THE EFFECTS OF CHRONIC ALCOHOL CONSUMPTION ON DERMAL PENETRATION OF PESTICIDES.

The skin is the major source of xenobiotic exposure in occupational settings. Topically applied ethanol is commonly used as a dermal penetration enhancer. The hypothesis of this work is that ethanol, consumed orally, will also behave as a dermal penetration enhancer. A series of four pesticides, parathion (MW = 227, log Kow = -4.5), 2,4-D (MW = 221, log Kow = -2.3), atrazine (MW = 216, log Kow = -2.3) and trifluralin (MW = 335, log Kow = -2.1) were selected as model compounds to test the effects of oral ethanol consumption on dermal penetration. These compounds were chosen for their diverse octanol water partition coefficient values (Kow). Male and female rats were fed a diet of 36% ETOH (25 mM in blood) or control diet for 4-8 weeks. The animals were then sacrificed, their skin shaved, brushed and placed in an in vitro Franz cell apparatus. The results of this study provide evidence that the acute oral ethanol consumption in rats results in increased penetration of pesticides into the skin. These studies imply that alcoholic workers have greater dermal exposure of chemicals than previously estimated.

816 TRANSCRIPT PROFILING OF MURINE LYMPH NODE CELLS: ALLERGEN-INDUCED EARLY GENE CHANGES.
C. J. Berta, J. G. Morgan, K. C. Cuddick, M. Cumberbatch, G. Orphanides, R. J. Dearman, G. E. Gerberick, C. A. Ryan and K. Kimbell. Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom and The Frontier & Gamble Company, Cincinnati, OH.

In order to identify early events induced in lymphoid tissue following topical treatment of mice with contact allergens, gene expression changes in draining lymph node cells (LNC) have been profiled using DNA microarrays. BALB/c strain mice were transplanted with THC and BA, and then harvested at 24 hours. The results of this study confirm the results of a pilot study performed using the same methods but different chromographic conditions.
were exposed topically to the potent skin sensitizer 2,4-dinitrofluorobenzene (DNFB; 0.5%) on the dorsum of both ears. Control mice were exposed to vehicle alone. Tumor lesions and the mRNA prepared. Radioiodinated cDNA probes were generated from mRNA samples and hybridized to in-house nylon microarrays featuring cDNAs of 4734 murine gene sequences arrayed on nylon chips (3356 assigned to known genes, 5398 expressed sequence tags (ESTs)). Changes in gene expression were calculated as the ratio of values for LNC isolated from DNFB-treated animals compared to LNC from concurrent controls (changes of 1.5 fold or greater were considered significant). Increases in expression were analyzed by 48 hours post exposure to DNFB exposed provided examples of both up- and down-regulated genes, including zinc and guanine binding protein-2, and a more extensive time course (18 to 120 hrs) using Northern blotting and/or RT-PCR analyses, indicating that changes in gene expression of G1 and G1-1, zinc and guanine binding protein-2, and G1 possibly G2 are robust markers of early changes in the lymph node provoked by contact allergen. In subsequent experiments the selectivity of these markers for contact allergies will be investigated.

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SUBCHRONIC TOXICITY OF 2, 3, 7, 8- TETRACHLOROBENZENO-P-DIOXIN (TCDD) OBEYS HABER'S C T R U LE AFTER ORAL ADMINISTRATION.

M. Lebodski', C. R. Crouch and K. R. Rozman. 1. * Physiology, Toxicology and Therapeutics, KU Medical Center, Kansas City, KS and 2. Section of Environmental Toxicology, GSP-Institut für Toxikologie, Neuberg, Germany.

Groups of 8 to 11: All mice were administered a loading dose of 100, 150 or 200 microg/kg TCDD following a biweekly maintenance doses of 150, 22.5, or 30 microg/kg by gavage in corn oil. A positive control group (n=10) received a single dose of urethane (1 mg/kg). All positive controls were injected with 4 microg/kg acrylamide into the urethane at 7 days, 5 days, and 2 days after treatment of urethane and 2 days after treatment of TCDD. The mean of these doses was 2.5 microg/kg into the urethane at 7 days, 5 days, and 2 days after treatment of TCDD. The mean of these doses was 2.5 microg/kg into the urethane at 7 days, 5 days, and 2 days after treatment of TCDD. The mean of these doses was 2.5 microg/kg into the urethane at 7 days, 5 days, and 2 days after treatment of TCDD. The mean of these doses was 2.5 microg/kg into the urethane at 7 days, 5 days, and 2 days after treatment of TCDD.

2, 3, 7, 8-TETRACHLOROBENZO-P-DIOXIN (TCDD) EXPOSURE OF MALE MOUSE UROGENITAL SINUS IN EXPONENTIAL CULTURES IMPAIRS PROSTATIC BUD FORMATION.

D. C. Upson, T. M. Lin, N. T. Rasmussen and R. E. Peterson. School of Pharmacy, Univ. of Wisconsin, Madison. WI.

* in vitro TCDD exposure on Gestation Day (GD) 13 impairs prostatic bud formation from the urogenital sinus (UGS) of the C57Bl/6J mouse fetus on GD 14. Ovulation of follicles by GD 14 UGS explants in organ culture is considered a marker of 5 days of exposure to TCDD, and (2) whether the inhibition of inhibition of budding, if observed, was AhR-dependent. Prostatic bud formation from the fetal mouse UGS is androgen-dependent. On GD 14 the UGS from wild-type and AhR knock-out (AhRKO) C57Bl/6J male fetuses were removed and cultured in 5% charcoal-treated FBS supplemented with either vehicle (Control), 10^-6 M testosterone (T), or 10^-6 M testosterone and 10^-5 M TCD (T+TCDD). Media was changed every 48 hrs. UGS explants were harvested after 5 days in culture and examined for prostatic bud formation by scanning electron microscopy. In Control UGS explants derived from both wild-type and AhRKO fetuses only 2 dorsal buds and no ventral buds were observed. This lack of prostatic bud formation was consistent with previous studies showing that this is an androgen-dependent process. Thus, in the absence of androgen stimulation virtually no buds were observed in either of the two Control groups (wild-type and AhRKO). Supplementation of the culture medium with 10^-5 M TCD produced buds in both genotypes, wild-type and AhRKO. In UGS explants exposed to T+TCDD the effect on budding was genotype-dependent. In wild type UGS explants exposed to T+TCDD prostatic bud formation resembled that of Control wild type UGS explants that received no androgen stimulation.

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PROTECTION BY VITAMIN E SUCCCINATE OF TCDD-INDUCED OXIDATIVE STRESS IN RATS.

E. Hassoun and A. Abu Mahfouz. Pharmacology, The University of Toledo. Toledo, OH.

TCDD is known to induce oxidative stress in hepatic, as well as brain tissues of rats after acute, subchronic and chronic exposures. In this study we demonstrate that subacute treatment of rats with TCDD can also induce oxidative stress in the same tissues, and that vitamin E succinate can provide protection against this effect. Animals were assigned to four different groups, including a control (C), TCDD-treated (T-treated), vitamin E succinate-treated (E-treated) and TCDD plus vitamin E-treated (T+E-treated) group. All treatments were given daily by gavage, beginning on GD 20 and continuing until GD 27. The T-treated group received 100 microg/kg TCDD/kg/day, while the E-treated group received 100 microg/kg TCDD/kg/day, plus 50 mg/kg vitamin E/kg/day. The T+E-treated group received 100 microg/kg TCDD/kg/day, plus 50 mg/kg vitamin E/kg/day. The T+E-treated group received combined dose regimen of the T and E-treated groups, and control group received corn oil vehicle used to dissolve TCDD and vitamin E succinate. Animals were sacrificed at the end of the treatment period and brain and hepatic tissues were assayed for various biomarkers of oxidative stress including production of superoxide anion (SA), lipid peroxidation (LP) and DNA damage. While subacute treatment with TCDD produced significant production of SA and LP, with significantly more effects produced in hepatic as compared with brain tissues, it didn’t produce significant DNA damage in either tissues. The results also indicate that vitamin E succinate provides significant protection against TCDD-induced SA and LP production in hepatic and brain tissues, with significantly more protection provided in brain as compared with hepatic tissues.

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LOW DOSE PRENATAL DIOXIN EXPOSURE IN RATS INDUCES SEXUALLY DIMORPHIC CHANGES IN OPERANT BEHAVIOR AND BRAIN MORPHOLOGY.


Prenatal exposure to high doses of TCDD (2, 3, 7, 8- tetrachlor dibenzo- p-dioxin) induces changes in motor activity and cognitive and sexual behavior. In an earlier study, prenatal exposure in rats to a single low dose of TCDD on gestational
day 18 (GD18) produced a significant dose-related reduction in wheel running op-erant behavior. In addition, a quantitative study of brain morphology showed that TCD2 also altered normal patterns of sexually dimorphic asymmetries in cortical thickness. The current study examined exposure to low, environmentally relevant levels of TCD2 at an earlier stage of gestation. It employed two endpoints: sched-ule-controlled operant behavior based on lever-pressing for food reinforcement, and cell counts in selected areas of cerebral cortex. Pregnant Sprague-Dawley rats were given a single ip, p.o., dose of 0, 20, 60, or 180 ng TCD2/kg on GD 8. Male-female pairs of offspring 90 days old from all dose groups were trained on two oper-ant procedures: (1) an incremental fixed ratio (FR) schedule, and (2) a multiple FR/differential reinforcement of low rate schedule (multi FR/ORL). TCD2 evoked sexually dimorphic response patterns in both procedures. Exposed males responded at lower rates than male controls, while exposed females responded at higher rates than their controls. Cortical cell counts from areas 17, 18a and 39 in brinaga -5.9 from offspring exposed to 180 ng/kg, the only dose examined so far, exhibited signif-icant reversal of hemispheric dominance in both males and females. These data demonstrate that prenatal exposure to TCD2 evokes sexually dimorphic patterns of operant behavior and cortical asymmetry in a manner consistent with previous reports. (Funded by ES08958 and ES10247 from NIEHS.)

822 EFFECTS OF TCDD, 2,3,4,7,8-PCDF AND PCB 126 ON ESTROGEN HYDROXYLATION IN MALIGNANT (MCF-7) AND BENIGN (MCF-10A) HUMAN MAMMARY TUMOR CELLS.

M. B. van Daarsen, J. T. Sanderson and M. van den Berg, Institute for Risk Assessment Sciences (IRAS), University Utrecht, Utrecht, Netherlands.

Mounting evidence suggests a role for estrogen metabolites in the etiology of breast cancer. In human breast tissue, estrogens are mainly metabolized by cytochrome P450 1A1 (CYP1A1, 7,8-epoxidenzyme) and P450 1B1 (CYP1B1), estrogen 4- hydroxylase. In several studies, the 4-hydroxylated but not the 2-hydroxylated metabolites of estrogens were found to be carcinogenic. This study investigated the effects of several dioxin-like compounds on estrogen hydroxylase activity in human mammary tumor cell lines MCF-7 (adenocarcinoma) and MCF-10A (fibrocytic disease). Cell cultures were exposed to various concentrations of 2,3,4,7-TCDD, 2,3,4,6-PCDF or PCB 126. After 72 hours the media were replaced with media containing 1 mM estradiol. Estrone-2- and 4-hydroxylase activities were de-termined by measuring 2- and 4-methoxyestradiol levels by GCMS. Both the constitutive and induced estrogen hydroxylase activities were lower in MCF-10A than in MCF-7 cultures. This was also true for ethoxyresorufin O-deethylase (EROD) activity in both cell lines. In MCF-7 cells, estrogen metabolism was induced by a dose-dependent manner with relative potencies in the order TCDD > PCDF > PCB 126, conform the WHO TEF values. Induction of estrogen metabo-lism was mainly attributed to induction of 2-hydroxylase of estradiol. In MCF-10A cells, only TCDD induced estrogen metabolism, markedly, while PCDF and PCB 126 appeared to have only a moderate inhibitory effect. Induction of estrogen hyd-roxylase by TCDD mainly resulted in elevated levels of both 2- and 4-hydroxylated metabolites of estrone. These data suggest a difference in response to dioxin-like compounds between malignant and benign mammary tumor cell lines. Studies on the effects of several other dioxin-like compounds on estrogen hydroxylase activity and mRNA levels of CYP1A1 and CYP1B1 in MCF-7 and MCF-10A cells are in progress.

823 TCDD-INDUCED ABNORMAL DEVELOPMENT OF PAWS IN TCDD-RESPONSIVE LACZ TRANSGENIC MICE.

N. E. Alejandro, D. A. Nazarenko and T. A. Gasiewicz, Environmental Medicine, University of Rochester, Rochester, NY.

The aryl-hydrocarbon Receptor (AhR) is a ligand-dependent transcription factor that mediates the toxicity of halogenated aromatic hydrocarbons including 2,3,7,8-tetrachl-robenzox-p-dioxin (TCDD). These compounds are potent reproductive and developmental toxicants due to their ability to modulate gene expression and cellular differentiation/proliferation. Determination of tissue-specific AhR transcriptional activity in vivo will allow the identification of tissues/cells where the AhR may have some functional roles susceptible to disruption by TCDD exposure. We have developed a transgenic mouse model containing an AhR-responsive reporter that can delineate the temporal and spatial activity of AhR in cell/region-specific manner. This model has identified several target tissues/cells during gestational day 13 to 16 including the lung, liver, genital tubercle, and paws after 24h TCDD exposure. Most widespread induction is in tissue that have been previously identified as target tissues, with the exception of the paw, a novel target. We hypothesize that TCDD-induced AhR activity in developing paws of mice is as-sociated with alterations in gene expression and aberrant development. Pregnant fe-males were exposed to TCDD (30 1E12) on GD 14. Twenty-four hours later and on post-natal day (PND) 2 paws were evaluated at a morphological and gene ex-pression level. Significant differences were identified upon morphometric analysis of the paw at PND 2 in both epibulbar cell layer and digit thickness, with TCDD exposed tissues being increased in both cases. Preliminary microarray analysis identified novel genes as well as known dioxin-responsive genes (CYP1B1) in the paws of day 12 embryos that were affected by TCDD exposure. The results indicate that the model is capable of detecting AhR activation, thus allowing the identification of developmental cell and gene targets of TCDD. (Funded by NICHD grant ES0943, Training grant 07026, and Center grant 01247.)

824 EFFECT OF TCDD ON THE SURVIVAL OF BONE MARROW-DERIVED DENDRITIC CELLS IN VITRO.

C. J. Funatke, C. E. Ruby and N. L. Kerkering, Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR and Environmental Health Science Center, Oregon State University, Corvallis, OR.

Previous studies in our laboratory have shown that an immunosuppressive dose of TCDD quantifies the absence of antigen-activates splenic dendritic cells (DC) in vivo. However, recovery of DC was subsequently reduced approximately 40%. We hypothesized that TCDD sensitizes DC to Fas-mediated apoptosis. To demonstrate a TCDD effect on DC survival, we used an in vitro bone marrow-derived dendritic cell (BMDC) model. On day 0, cells from the bone marrow of female from C57Bl/6 mice were cultured in media containing 20% FBS and GM-CSF for 6 days. TNF-α was added on day 6 to generate a mature DC phenotype. On day 7, media and cytokines were removed and replaced with media containing 5% FBS and vehicle or DC. On day 7, agonistic anti-Fas (Jo-2) or control IgG antibodies were added to the cultures. On the cultures, the cells were assayed for apoptosis using Annexin-V-PI (fluorescent flow cytometry). In separate experiments, TCDD alone increased apoptosis or enhanced the degree of apoptosis induced by Fas ligation. In all experiments, the highest degree of apoptosis was seen when TCDD (10M M) and anti-Fas were combined. In order to determine if the increase in apoptosis was due to increased levels of Fas on the cell surface, we looked at the level of Fas expression on BMDC by flow cytometry. We found no difference in Fas expression between vehicle- and TCDD-treated cells, suggesting that TCDD may alter intracellular signaling pathways leading to apoptosis. Further studies are necessary to determine if the relationship between TCDD and Fas signaling is ad-ditive or synergistic. Supported by NIH Grants P01 ES00040 and P01 ES07060.

825 TIME-DEPENDENCE OF TCDD- AND HXCD-INDUCED CYP 1A1 EXPRESSION AS MEASURED BY EROD ACTIVITY, WESTERN AND NORTHERN BLOTS.

C. R. Crouch, M. Lebofsky, A. DeZoyas, D. S. Soul, K. W. Fried and K. K. Romman, Department of Pharmacology, Toxicology and Therapeutics, KU Medical Center, Kansas City, KS. Section of Environmental Toxicology, GSF-Institute for Toxicology, Neuruppin, Germany, and 1Muskie College, Bethlehem, PA.

Female Sprague-Dawley rats (-250 g, Harlan) were housed in large polycarbonate cages with hardwood bedding. Feed and water were provided ad libitum to all ani-mals. Rats were given a loading dose of either 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) or 1, 2, 3, 4, 7, 8-hexachlorodibenzo-p-dioxin (HxCD) in corn oil via oral gavage (five animals per treatment group). Animals were dosed once daily, 10 days. Animals were sacrificed on days 1, 3, 5, 7, 10, and 14, respectively, to maintain pharmacokinetic steady state. Three i.p. effective dose of TCDD/HxCD (0.0125/0.3125, 0.05/0.125, or 0.25/0.5 microg/kg) were selected based on their toxic equivalency. Rats were terminated, 2, 8, 32, or 64 days after receiving the loading dose. Livers were re-moved, weighed and snap frozen in liquid nitrogen. TCDD caused a dose- and time-dependent induction of EROD activity. There was about a 100-fold increase in EROD activity at constant time (day 2) and about an additional 5-fold increase at constant dose (day 64). The same samples that were used to measure EROD activity were also analyzed for CYP 1A1 mRNA and protein by Western and Northern blotting, respectively, for Western blotting, a rabbit anti-rat CYP1A1 polyclonal antibody from XenoTech (Kansas City, KS) was used. Murine CYP1A1 cDNA was obtained from ATCC for use in Northern blots. Semi-quantification of Western and Northern blot results were obtained using Gel Pro image analysis. Both CYP 1A1 mRNA and protein increased in a time-dependent manner in both the TCDD- and HxCD-treated rat livers, in agreement with the induction of EROD activity. The CYP 1D1 isozyme which was induced was approximately 2-fold between 32 and 64 days at constant dose there was a greater (approximately 8-fold) increase in EROD activity in the same samples.

826 DIOXIN IMPAIRS OSMOREGULATION IN THE ZEBRAFISH LARVA.

S. M. Belbo, W. Heideman and B. E. Peterson, School of Pharmarcy, University of Wisconsin, Madison, WI.

The most prominent sign of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) developmen-tal toxicity in the zebrafish larva is edema. However, the cause of the edema and its relation to the other signs of TCDD toxicity in zebrafish is unknown. One
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**EFFECTS OF IN UTERO / LACTATIONAL EXPOSURE TO TCDD ON BONE IN RATS.**

M. Viiksluksel, T. Jämäät, H. M. Kattainen, U. Simanainen, J. T. Tuomisto, and E. J. Titunikainen. 1Department of Environmental Health, National Public Health Institute, Kuopio, Finland; 2Department of Medical Technology, University of Oulu, Oulu, Finland; and 3Department of Anatomy and Cell Biology, University of Oulu, Oulu, Finland.

We have previously shown that TCDD treatment alters bone geometry and decreases mechanical strength in adult rats. In this study bone geometry, mineral density, and mechanical strength were examined in rats exposed to TCDD in utero / lactationally. Pregnant female rats of TCDD sensitive line C were given a single oral dose of TCDD (0.6, 0.03, 0.1, 0.5 or 1 μg/kg) on gestational day 15, and female offspring were examined on postnatal day 30. Tibial and femoral diaphyses were scanned using a peripheral quantitative computed tomography (pQCT) system. Mechanical properties of tibiaal and femoral shaft and femoral neck was assessed using three-point bending test and axial loading, respectively. Body weights of offspring were slightly decreased at the highest dose-level only. Tibial and femoral length, as well as diaphysial cortical and medullary cross-sectional area were significantly decreased. In addition, cortical mineral density and polar moment of inertia were decreased. Some of these changes were dose-dependent, but reached statistical significance only at 1 μg/kg TCDD. Tibiaal mechanical testing revealed significantly decreased bending breaking force and bending stiffness of tibiaal and femoral shaft, and femoral neck. These changes were only seen at the highest dose level. The results indicate that bone is a sensitive target of dioxin toxicity, and that pre- and neonatal rats are clearly more sensitive to the bone effects than adult rats. Supported by the Academy of Finland, the Finnish Research Programme on Environmental Health, Project 42551, and the European Commission, Contract QLK4-CT-1999-01446.

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**ADULT TCDD EXPOSURE AND EFFECTS ON MALE REPRODUCTIVE ORGANS IN THREE DIFFERENTIALLY TCDD SENSITIVE RAT LINES.**

U. Simanainen,2,4 H. Kattainen,4 A. Adamson,4 J. Tuomisto,4 J. Trippal,4 and M. Viiksluksel.1 1Laboratory of Toxicology, National Public Health Institute, Kuopio, Finland; 2University of Kuopio, Kuopio, Finland; and 3Dept of Physiology, University of Turku, Turku, Finland.

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) exposure during adulthood is known to have adverse reproductive effects. The exceptional resistance of the Han/Wistar (Kuopio) strain to acute lethality of TCDD is independent of two different genes: the H/W-type mutated AhR and the 2,7,8-trichlorodibenzofuran (TCDF) resistant strain. In this study, the role of the resistance genes in TCDD toxicity was investigated in the two different strains of these two different genes: The H/W-type mutated AhR and the 2,7,8-trichlorodibenzofuran (TCDF) resistant strain. In this study, the role of the resistance genes in TCDD toxicity was investigated. In conclusion, these data suggest that the resistance genes play a significant role in the toxicity of TCDD.

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**17ß-ESTRADIOL METABOLISM AND EROD ACTIVITY IN RAT LIVER AND LUNG AS BIOMARKERS OF CHRONIC EXPOSURE TO TCDD AND RELATED COMPOUNDS.**


The expression of cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1) represents sensitive biomarkers of exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and related halogenated aromatic hydrocarbons (HAHs). CYP1A1 mediated chloroethylnitrosurea O-dealkylase (EROD) and estradiol metabolism were assessed in liver and lung microsomes from female Sprague-Dawley rats following 13 weeks of daily oral (gavage) exposure to TCDD, 2, 3, 4, 7-Fe(II), PCB 126, or PCB 153. Estradiol-4-hydroxylation (2-OHE2) activity, a measure of human CYP1B1 expression, is currently being evaluated as a rat model of CYP1B1 expression. In addition, estradiol-2-hydroxylation (2-OHE2) activity was measured because of its significant role in estradiol metabolism. TCDD exposure produced a dose related increase in these three activities which, with the exception of 2-OHE2 in liver, were significantly increased at an exposure of 3 μg/kg/d. Tissue-specific differences were noted with lung showing a greater fold induction in 4-OHE2 and 2-OHE2 activities, while liver displayed a greater increase in EROD activity. With the exception of PCB133 (TEF=0), each of the HAHs increased EROD, 4-OHE2, and 2-OHE2 activities in a dose-related manner, which generally supports the current Toxic Equivalency Factors (TEF) for these compounds. Dose-response relationships following chronic (TCDD, PCB126, PCB 153) as well as acute (PCB 126, PCB 153) exposure will also support the additive activity of these agents, based on the current TEFs for these HAHs. These studies were carried out using tissues provided to us by the National Toxicology Program as part of a series of chronic 2-year rat bioassays examining the relative carcinogenic potencies of dioxin-like compounds. Following completion of the cancer studies, it will be
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ZEBRAFISH FIN REGENERATION AS A MODEL FOR DEVELOPMENTAL TOXICITY OF 2, 3, 7, 8-
TETRACHLORODIBENZO-P-DIOXIN (TCDD).

J.M. Zdorov and R.L. Tanguay, School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO.

Adult zebrafish completely regenerate their caudal fins following amputation. Fin regeneration can now be followed in vivo and for the first time, can be used to study developmental processes. 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is known to cause toxicity by binding to the AhR (aryl hydrocarbon receptor), which is the nuclear receptor responsible for the transactivation of a panel of genes in the body. These genes are involved in the development of cancer. The study found that TCDD impaired regeneration at multiple stages of regeneration. Effects of TCDD on zebrafish were studied at various time points during regeneration. They found that TCDD impaired fin regeneration at multiple stages of regeneration. Environmental exposures to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) always occur as part of a complex mixture. In order to assess the potential risk associated with these mixtures, the Toxic Equivalency Factor (TEF) method was developed, and is used to estimate the contribution of each compound to the overall toxicity of the mixture. However, the results of this study were supported by EPA CP920208.

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COMPARING MIXTURES OF DIOXIN-LIKE AND NON-
DIOXIN-LIKE PCBs TO TCDD.

D. E. Burgin, J.L. Damien, and L.S. Bittman, 1Toxicology, UNC Research Triangle Park, NC, 2ETDP, USDA, Research Triangle Park, NC, and 3HSD, USEPA/NHEERL, Chapel Hill, NC.

The tissue distribution of dioxin is dose-dependent due to hepatic sequestration by CYP1A2, which is an inducible binding protein. This is the method of dioxin and chlorinated phenols. CYP1A1 is dependent on the ability to induce the activity of the enzyme and inhibit human and rat CYP1A2 activity. Rat and human CYP1A2 are known to be important in the presence of many xenobiotics. CYP1A2 is important in the presence of many xenobiotics. CYP1A2 is a member of the cytochrome P450 superfamily and hydroxylates many xenobiotics.

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INHIBITION OF HUMAN AND RAT CYP1A2 BY TCDD
AND DIOXIN-LIKE CHEMICALS.

D. F. Strobl, L.S. Bittman,3 Bittman, and M.J. DeVoit, 1Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, NC and 2ETTD, NIEHS, Research Triangle Park, NC.

The tissue distribution of dioxin is dose-dependent due to hepatic sequestration by CYP1A2, which is an inducible binding protein. This is the method of dioxin and chlorinated phenols. CYP1A1 is dependent on the ability to induce the activity of the enzyme and inhibit human and rat CYP1A2 activity. Rat and human CYP1A2 are known to be important in the presence of many xenobiotics. CYP1A2 is important in the presence of many xenobiotics. CYP1A2 is a member of the cytochrome P450 superfamily and hydroxylates many xenobiotics.

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EFFECT OF CHLORINATED PHENOTHIAZINES ON
OVULATION IN RATS.

K.W. Fried,2 X. Cao,3 B.K. Petroff,2 K.W. Schramm,2 P.F. Tarranezen,2 and K.K. Rozan,1 Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, 2Section of Toxicology, GSP Institute for Ecotoxicology, Neuberg, Germany, 3Center for Reproductive Science, University of Kansas Medical Center, Kansas City, KS, and 4Office of Science and the Environment, University of Kansas Medical Center, Kansas City, KS. Sponsor: K.K. Rozan.

Adult female Sprague-Dawley rats were administered 1mg/kg/day of tetra-
chlorinophenol sodium (TCP) or its sulfoxide (TCP-O) in glycerol (4ml/kg). The study was performed to obtain data for the determination of the potency of TCP-O relative to TCP-O for a variety of endpoints. Male and female Sprague-Dawley rats (10/sex/group) were administered TCP-O (100%) and their tissues were examined for toxicological endpoints. The study was performed to obtain data for the determination of the potency of TCP-O relative to TCP-O for a variety of endpoints. Male and female Sprague-Dawley rats (10/sex/group) were administered TCP-O (100%) and their tissues were examined for toxicological endpoints. The study was performed to obtain data for the determination of the potency of TCP-O relative to TCP-O for a variety of endpoints. Male and female Sprague-Dawley rats (10/sex/group) were administered TCP-O (100%) and their tissues were examined for toxicological endpoints. The study was performed to obtain data for the determination of the potency of TCP-O relative to TCP-O for a variety of endpoints. Male and female Sprague-Dawley rats (10/sex/group) were administered TCP-O (100%) and their tissues were examined for toxicological endpoints.
Immature female rats were administered 1 and 2 mg/kg/day of TCPT or TCPT-O in xylene (4 ml/kg) for 4 days. Equine chronic gonadotropin (eCG; SIU) was administered 24 h after the first dose rate to induce follicular development. The animals were terminated at 72 h after eCG injection, ovaries harvested and the number of ova scored. There was an insignificant decrease (about 50%) in the number of ova among those treated with the TCPT-treated rats. However, TCPT-O dose-dependently and at the highest dose completely inhibited ovulation. In addition, ethoxyresorufin-O-deethylase (EROD) was determined in the livers of all rats. There was a very slight induction of EROD with both compounds at the highest dose. The structure of TCPT was elucidated by UV-VIS spectroscopy, HPLC, NMR spectroscopy and by GC/MS. Single crystal X-ray spectroscopy provided the final proof for the structure of 1, 3, 7-TCPT.

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2, 3, 7, 8-TETRACHLORIDIBENZO-P-DIOXIN INHIBITS TRANSCRIPTIONAL ACTIVITY OF THE 3α ENHANCER IN LIPOPOLYSACCHARIDE-STIMULATED CH12.LX B-CELLS.

C. E. Sulecic, Y. J. Na, J. H. Ostreich, M. P. Hoapple and N. E. Kaminski. Pharmacology & Toxicology, Michigan State University, East Lansing, MI; Korea Advanced Institute of Science and Technology, Daejon, South Korea and Dow Chemical Company, Midland, MI.

The B-cell is a sensitive cellular target of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) with the inhibition of the antibody forming cell response being a hallmark phenotype of TCDD toxicity. The actual molecular mechanism responsible for this altered B-cell function by TCDD is unclear. Our previous results describing the CH12.LX (AhR-expressing) and BCL-1 (AhR-deficient) B-cell lines have supported an AhR/dioxin-responsive (Adr) and dioxin-responsive (DRE)-mediated mechanism for TCDD-induced inhibition of Ig heavy chain expression and thus of immunoglobulin (Ig) M secretion. Transcriptional activation of the Ig heavy chain genes involves several regulatory elements including the 3α enhancer, which is composed of four regulatory domains. One of these domains, 3α-hex, contains a DRE overlapping a κB motif. We have previously demonstrated binding of the AhR nuclear complex and NF-κB proteins to these motifs. In the present study, a luciferase reporter gene containing only the 3α-hex domain or the full-length 3α enhancer was transiently transfected into the CH12.LX cells to determine the effect of TCDD and LPS on the activity of these enhancers. The co-transfection of TCDD and LPS had the greatest effect on these enhancers compared to either treatment alone. Interestingly, the co-transfection of TCDD and LPS markedly induced the activity of the 3α-hex domain, whereas LPS alone induced the full-length 3α enhancer but this induction was markedly inhibited with the addition of TCDD. Induction of the 3α-hex domain by the co-transfection of TCDD and LPS may regulate the full-length 3α enhancer leading to inhibition of Ig heavy chain expression. (These studies were supported by NIH ES05220 and NEHS 3F32ES05994-01)

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NON-COPLANAR POLYCHLORINATED BIPHENYL (PCB) CONGENERS INHIBIT PCB-INDUCED ACTIVATION OF THE 3α-H54 ENHANCER IN LIPOPOLYSACCHARIDE-STIMULATED CH12.LX B-CELLS.

Y. J. Na*, C. E. Sulecic, K. Yang and N. E. Kaminski. Pharmacology & Toxicology, Michigan State University, East Lansing, MI; Korea Advanced Institute of Science and Technology, Daejon, South Korea.

Polychlorinated biphenyls (PCB) are ubiquitous contaminants in the environment and many of their toxic effects including inhibition of B-cell differentiation are elicited by binding to the aryl hydrocarbon receptor (AhR). However, non-co-planar, ortho-chlorinated PCB congeners have been shown to inhibit AhR agonistemulated responses, such as inhibition of Ig heavy chain expression and immunoglobulin (Ig) M secretion. Transcriptional regulation of the Ig heavy chain gene involves several regulatory elements including the 3α-hex domain, which is composed of four regulatory domains. One of these domains, 3α-hex, contains a DRE overlapping a κB motif. In transient transfection experiments, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) enhanced the activity of a luciferase reporter gene containing the 3α-hex enhancer domain in lipopolysaccharide (LPS)-activated CH12.LX B-cells. The purpose of the present studies was to determine the effects of addition of LPS to 125B cell cultures, alone or in combination with the non-co-planar PCB 153 (2, 3, 4, 5, 5) 5 or PCB 52 (2, 3, 4, 5, 5) on the activity of the 3α-hex enhancer domain in LPS-activated CH12.LX cells. Similar to TCDD, PCB 126 enhanced the LPS-induced activation of the 3α-hex domain. In addition, PCB 153 and PCB 52 reversed the effect of PCB 126 on 3α-hex activity. Activation of the 3α-hex domain by PCB 52 may alter the normal activation pattern of the 3α enhancer leading to inhibition of Ig heavy chain expression in addition.

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ALTERED GENE EXPRESSION AND CUTANEOUS TOXICITY.

P. Gobstier and J. Kimbrel. Poster & Gamble, Cincinnati, OH and Syngenta, Cheshire, United Kingdom.

Adverse skin reactions can take a variety of forms and be induced by many biologically independent mechanisms. Recently, important progress has been made in defining the molecular basis for skin sensitization, skin irritation and other forms of cutaneous toxicity. In particular, the application of transcript profiling and related technologies has allowed the characterization of altered patterns of gene expression that collectively provide important new insights into the relevant toxic mechanisms. Moreover, it is anticipated that the association of novel genes with particular forms of dermal toxicity will yield exciting new opportunities for hazard identification. This proposed Symposium brings together the first time experts in skin biology and cutaneous toxicology to review how characterization of changes in gene expression are providing new insights into mechanisms of toxicity and opportunities for new approaches to hazard assessment. This Symposium is particularly timely because speakers will be describing the application of transcript profiling and proteomic analysis to monitor changes in gene expression and protein production.

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INDUCTION OF SKIN SENSITIZATION: GENE EXPRESSION PROFILES.

R. J. Deerman and J. Kimbrel. Reinsch, Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom.

An increasingly sophisticated appreciation of the cellular and molecular events associated with skin sensitization, linked with the availability of methods for transcript profiling, provides an opportunity to design alternative approaches to toxicological evaluation based on induced changes in gene expression. A central event in the induction of contact allergy is the induction of lymph node cell (LNC) proliferation, a response that forms the basis of the kinetic lymphoid node assay (KLNA), a method used for the identification and characterization of skin sensitization hazard. We have now examined whether changes in gene expression patterns induced selectively by antigen exposure can be identified using DNA microarray technology. Gene expression changes have been assessed following topical exposure of BALB/c strain mice to the contact allergen 2, 4-dinitrofluorobenzene (DNFB). In parallel investigations, transcript profiling has been performed on human blood derived dendritic cells (DC) cultured in the presence of non-toxic concentrations of
DNFB. Custom DNA microarrays comprising 8734 marine genes (3336 of which are assigned to known genes) or approximately 12500 human genes, each arrayed in duplicate, were utilized. In both cases, the majority of genes was unaffected by treatment with DNFB. Modest DNFB-induced changes in gene expression patterns of human DC were recorded, however, using the current culture conditions, these appear to be largely donor-dependent. Robust changes (up- and down-regulation) in the expression of mRNA for a small number of genes were observed following topical treatment of BALB/c strain mice with DNFB, with annexin and glycogen-synthesizing-dependent cell adhesion molecule-1 (GlyCAM-1) being the strongest up- and down-regulated genes, respectively. These genes may provide for robust and relatively sensitive markers of early changes induced by contact allergens.

**840 ALTERED KERATINOCYTE GENE EXPRESSION FOLLOWING TREATMENT WITH IRRIITANTS.**

E. Corinigi and C. L. Galli, Pharmacological Sciences, University of Milan, Milan, Italy.

In the screening of topical drugs, cosmetics and other chemicals for human use, it should be very important, both from safety and economic point of view, to have biological markers to discriminate irritant and allergic contact dermatitis that have different impact on human health. Due to their anatomical location, keratinocytes are among the first cells to be exposed to various toxicants and, the use of these cells as simplified in vitro model to evaluate the potential toxicity of chemicals destined for cosmetic application is amply justified. The identification of new target genes selectively modulated by skin toxicants offers the possibility to develop mechanism-based in vitro assay. We have data indicating the possibility to use the mRNA differential display technology (DD-PCR) and DNA microarray to identify differentially expressed genes following allergen or irritant treatment in commercially available reconstituted human epidermis (EpiDerm TM). Results identified adipose differentiation-related (ADRP) protein as up-regulated by both irritant and allergen, and KIAA0368 as selectively up-regulated by contact allergen. ADRP is a 53kDa membrane-associated protein and it is a marker of lipid accumulation, being an ubiquitously expressed lipid storage droplet-associated protein. The induction of ADRP is indicative of increased intracellular lipid deposits, which in turn can lead to cell degeneration and maybe also indicative of abnormal keratinization. On the contrary, KIAA0368 is probably an elongation factor involved in protein neosynthesis. It codes for a protein of apparent molecular mass >100kDa, located on chromosome 9 and ubiquitously expressed. Its role in skin irritation or allergy is at present unknown. These data indicate the enormous potential of functional genomic techniques, which allow the identification of genes not immediately connected with the immune response, or even novel genes with unknown functions.

**841 MOLECULAR CHANGES IN SKIN FOLLOWING ACUTE DERMAL EXPOSURES WITH JP-8 JET FUEL AND SOLVENTS.**

J. N. McDougal, Geo-Centers Inc. (AFRL/HEST), Air Force Research Laboratory, Wright-Patterson AFB, OH.

Many products and chemicals cause irritation when they contact the skin. Whole animal primary irritation testing (Draize test) has been around since 1944 with many modifications and variations. With acute tests, the barrier function of the skin is often disrupted and then the substance is placed in occluded contact with the skin for 24 hours and the responses are graded. Irritancy testing was designed to provide relative irritancy of substances, but these tests don’t provide useful information for risk assessments or industrial hygiene. In a real exposure situation, a substance would have to diffuse past the stratum corneum barrier down into the viable tissue to produce irritation. Since chemicals diffuse through the skin at different rates and have different irritant potencies, there is an exposure duration for most substances that would not cause irritation. Only minutes of contact could be safe for some substances but it might take hours or days for other substances to cause irritation. The purpose of these studies, using substances with different irritant properties, is to characterize the biological cascade in the skin that results from an acute irritating event. Ultimately our goal is to develop a biologically based model of irritation that can be used to predict safe exposure duration for a wide variety of compounds. We exposed the backs of rats to jet fuel, xylene, dimethyl and sodium lauryl sulfate for one hour and investigated the temporal changes in interleukin-1 alpha levels, inducible nitric oxide synthase levels and nitric oxide production. We also measured the time course of lipid peroxidation, oxidative stress and low molecular weight DNA levels in response to these substances. Traditional histology and immunohistochemistry were also used to compare response of the skin to the fuel and solvents. We found that the parameters in the irritant cascade that we investigated responded differently depending on the degree of irritation of each chemical. Further work will refine our preliminary understanding of this acute irritant cascade.

**842 ALTERED GENE EXPRESSION IN KERATINOCYTES FOLLOWING ARSENIC EXPOSURE.**

D. R. Germonces, Environmental Immunology, NIEHS, Research Triangle Park, NC.

Previous studies in our laboratory have suggested that arsenic modulates neoplastic disease in the skin by inducing overexpression of genes encoding specific cytokines and growth factors. We have completed in vitro studies which compare altered cytokine gene expression following exposure to either Arsenic III and Arsenic V or their monomethyl and dimethyl metabolites in normal human epidermal keratinocytes (NHEK) and HaCaT keratinocytes (an immortalized cell line commonly used in arsenic, oxidative stress, and epidermal research) following arsenic exposure. Pathway mapping, FACS analysis and viability studies also have been performed and the correlation between altered gene expression and physiological effects (e.g., cell cycle arrest, DNA damage, elevated cell proliferative responses, etc.) will be presented.

**843 OVEREXPRESSION OF MOUSE HOMOLOGUE OF HOS UBQUITIN LIGASE RECEPTOR IN SKIN TUMORS IMPLICATION IN CONSTITUTIVE ACTIVATION OF NF-kB.**


The mouse skin model of multistage carcinogenesis is an excellent model to study the critical role of specific gene expression in the induction of squamous cell carcinomas. We have found that the function of NF-kB is altered during the induction of skin cancer. NF-kB transcription factor is activated upon ubiquitination and subsequent proteolysis of its inhibitor IKB. The phosphorylation-dependent ubiquitination is mediated by IKB kinase (IKK). We identified a novel murine F-box/WD40 repeat containing protein, mHOS (a homologue of HOS/BTCP). mHOS efficiently binds Skil protein (a core component of SCF ubiquitin ligase), and phosphorylates IKB. We found that mHOS associates with SCF-ROC1 E3 ubiquitin ligase activity. We have also observed that mHOS is overexpressed in chemically-induced mouse skin tumors, and its overexpression (but not normal expression) coincides with the accelerated degradation of IKB in vivo. The role of mHOS in the constitutive activation of NF-kB in skin carcinogenesis will be discussed.

**844 DEFINING THE CELLULAR AND MOLECULAR MECHANISMS OF TOXICANT ACTION IN THE TESTIS.**

J. H. Reichburg and K. Borokheside, 'College of Pharmacy, The University of Texas at Austin, Austin, TX' and Pathology and Laboratory Medicine, Brown University, Providence, RI.

Despite the association of environmental toxicant exposure and testicular injury, there is little mechanistic data of the molecular and cellular pathways involved. This symposium is focused on the identification of novel molecular and cellular pathways that modulate the response to testicular toxicants. The recently discovered G-protein coupled receptor family member, flaminigo, mediates cell-cell adhesion and has been identified in the testsis. A specific subset of flaminigo homologues exhibits a reduced cell-specific expression at early time points after phthalate exposure suggesting that they may be proximal phthalate targets. Observations of the newly identified family of receptors (D3R, S & G) in the testsis and differential cellular changes in their expression after phthalate exposure suggest that they may act as surrogates or in concert with the widely described FAS-signaling pathway in the initiation of germ cell apoptosis. A variety of exposures to rodent (radiation, chemotherapy, toxins) and human (health care) testicular exposures using NEHEK immunocytochemistry and immunohistochemistry were used to explore response of the testsis to phthalates and phthalate metabolites. Lower testosterone and serum FSH levels allow for a re-initiation of spermatogonial development. Recent investigation of additional models of persistent atrophy such as knock out mice, the aged brown Norway rat, EDS-induced Leydig cell deficient rat, and primates, have broadened insight into the mechanisms responsible for persistent atrophy. cDNA arrays are a useful technique for examining changes in mRNA expression. A custom mouse tests
cDNA array has been developed that contains 950 genes. cDNA array analysis of testis mRNA from mice exposed to bromochloroacetic acid (BCA), a drinking water disinfectant, shows an alteration in 42 of 950 genes. Of these, a specific set of 10 genes was identified that represents proteins involved in cell adhesion complex. This strengthens the findings that BCA acts by disrupting spermogenesis and provides new insights into the cellular and molecular mechanisms by which BCA acts.

845 PURSUIT OF THE TESTIS PHALALATE TARGET: A NOVEL CADHERIN IS AN EARLY RESPONDER.

K. Johnson, S. Thompson and K. Bockelheide. Pathology and Lab. Medicine, Brown University Medical School, Providence, RI.

Phthalates are environmental pollutants found in soft plastics, and human exposure levels are significant. Phthalates target the testis, with toxicity features including early alterations in Sertoli cell morphology and biochemistry, disruption of Sertoli-germ cell adhesive contact, and altered Sertoli cell G-protein-stimulated cAMP generation. These endpoints indicate that a Sertoli-germ cell adhesion complex and a G-protein coupled receptor (GPRC) signaling pathway are early phthalate targets. Recently, GPCR family members termed phalmoins were discovered which mediate cell-cell adhesion, likely via extracellular cadherin domains. Thus, phalmoins appear to couple cell-cell adhesion to the stimulation of G protein-based signaling, making them attractive early phalalate targets. Using a degenerate RT-PCR cloning strategy, we discovered that all three phalmoins (1, 2, and 3) are expressed in testis with unique, cell-specific expression patterns. By analyzing phalmoins mRNA expression in postnatal testis, phalmoins 1 and 2 are likely Sertoli cell products while phalmoins 2 and 3 germ cell products. To determine if phalmoins biology is affected by phalalate exposure, the testis mRNA expression of these phalmoins was examined by semi-quantitative RT-PCR following exposure of 28 day-old rats by exposure to 1 g/kg mono(2-ethylhexyl) phalalate. Significant declines in the mRNA expression of phalmoins 1 and 2 were observed to 70% of control within 3 hrs with additional declines at later time points (35-50% of control at 6 hrs). However, the effect of phalalate was specific to phalmoins 1 and 2 GPRCs; the mRNA level of another Sertoli cell GPCR (the FSH receptor) was reduced somewhat at 3 hrs but recovered at 6 hrs post-exposure, while phalmoins 3 mRNA levels were elevated at 6 hours. These data indicate that phalmoins 1 and 2 respond early to phalalate exposure and lead to a model of testis phalalate toxicity in which a phalmoins-mediated Sertoli-germ cell adhesion/signaling complex is a proximal phalalate target, leading to germ cell degeneration and apoptosis.

846 NOT SO FAS: EVIDENCE FOR THE DIFFERENTIAL INVOLVEMENT OF FAS-INDEPENDENT SIGNALING PATHWAYS IN THE TESTIS.

J. H. Richburg. College of Pharmacy, The University of Texas at Austin, Austin, TX.

The Fas-FasL signaling system is proposed to be critical in the regulation of testicular germ cell apoptosis and maintenance of spermatogenesis. The Fas signaling system has been implicated in the initiation of germ cell apoptosis after exposure to the Sertoli cell toxicant mono(2-ethylhexyl) phalalate (MEHP). Interestingly, gld mice, which express a mutant form of FasL, are fertile and display normal spermatogenesis. In addition, a limited protection against MEHP-induced germ cell apoptosis was observed in gld mice. To evaluate the participation of other FasL death receptor signaling systems in the testis, we examined the expression of the newly described death receptors (DR-4, 5 and 6) in both C57BL/6 (B6) and gld mice after MEHP exposure (1/5 mg/kg). By western blot analysis, each of the DR's was present in B6 and gld mouse testis. A comparison of the pattern of protein expression of Fas to that of the DR's in B6 mouse after MEHP exposure revealed that Fas, DR4 and DR5 show similar patterns of expression whereas DR6 has a unique expression pattern. DR6 expression in gld mice was also strikingly different from that in B6 mice with an early increase in protein expression (3 h) that remained for the duration of MEHP exposure. The delayed induction of DR6 after MEHP exposure in the B6 testis suggests that this receptor may be activated as a secondary response to B6 germ cells to decreased hormonal, nutritive or physical support that results from MEHP-induced Sertoli cell injury. The early induction of DR6 in gld mice implies that the mechanism that leads to the induction of DR6 is already primed. The evaluation of NF-kB in the gld testis shows an early increase in NF-kB DNA binding similar to the changes in DR6 expression seen in these mice. Taken together, these findings indicate that the DR4, 5 or 6 systems in mice may be functioning in parallel with the Fas system to regulate germ cell apoptosis and/or that one or more of these DR's may act as a surrogate for the Fas system when it is either overwhelmed or dysfunctional. (Supported by NIEHS ES09145 & ES07786)

847 REVERSAL OF TOXICANT-INDUCED TESTICULAR ATROPHY: MODELS AND MECHANISMS.

H. A. Schoenfeld1, K. Bockelheide1, G. Sherry1 and M. L. Meistrich2. 1Pathology and Laboratory Medicine, Brown University, Providence, RI and 2University of Texas M.D. Anderson Cancer Center, Houston, TX.

In rodents, severe toxicant-induced testicular injury results in a persistent post-exposure atrophy with ongoing proliferation of stem germ cells and apoptosis of their progeny. The block to further spermatogonial development is relieved by hormonal manipulations that lower testicular testosterone and serum FSH levels. In the rat it appears that both the testicular testosterone and FSH levels are responsible for inhibiting spermatogonial development, whereas in a similar model of spontaneous spermatogonial failure, jcl mutant mice, it appears to be only testosterone. Any androgen appears to be inhibitory and acts through the androgen receptor. In some cases, a transient suppression of testosterone can result in maintenance or reinitiation of spermatogonial development for a period of time to restore complete spermatogenesis and fertility. However, therapy with the toxicant ethane dimethane sulphonate, which selectively ablates testosterone-producing Leydig cells, suppresses both serum testosterone and testicular testosterone yet fails to stimulate a reversal of 2, 5-hexanedione-induced injury, regardless of whether it is administered alone or in combination with GnRH agonist therapy. These studies suggest that paracrine-acting factors produced by Leydig cells may also be required for the reversal of testis injury. This paradigm of induction of otherwise "irreversible" injury, followed by hormonal manipulations to maintain spermatogenesis, is currently being tested in a non-human primate model. The results of this primate study will be discussed along with insights into the mechanism of atrophy reversal derived from ongoing investigations of rodent models.

848 GENOMIC ANALYSIS OF THE TESTICULAR TOXICITY OF HALOACETIC ACIDS.

D. J. Dix and J. C. Rockett. Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.

Gene expression profiles from testes of mice exposed to effective doses of reproductive toxicants can be used to identify genes and gene networks critical to male fertility, their toxic mode of action, and understand gene-environment interactions. A custom mouse cDNA microarray interrogating 950 genes was used to analyze gene expression in the testes of mice exposed to a halocetic acid (HA). HAs are unintended byproducts of drinking water disinfection. Bromochloroacetic acid (BCA) is a commonly occurring HA that acts as a reproductive toxicant in male rodents, targeting sperm and spermatids. Adult C57BL/6N male mice were dosed via gavage with 0, 8, 24, 72 or 216 mg/kg BCA once daily for 14 days. The mice were then used in a 40-day sequential breeding assay to determine if BCA negatively affects reproductive performance by targeting a particular phase of spermatogenesis. Decreased fecundity and infertility was observed during the first 10 days of the breeding assay, indicating that BCA was affecting post-meiotic spermatogenesis. Histological analysis of testes harvested at the end of dosing revealed spermatid retention and abnormal residual bodies, consistent with disruption of spermatogenesis (the process of spermatid separation from Sertoli cells). DNA array analysis of these testes indicated that expression of 42 of the 950 genes interrogated was significantly altered by the higher doses of BCA. Of the 42 altered genes, 10 were cell adhesion genes, 7 stress response genes, and 4 steroid metabolism genes. These 10 cell adhesion-signaling genes included vinculin, a catenin, an integrin and tubulin. These families of proteins are components of or associated with the extracellular matrix (ECM) which form cell-cell junctions between Sertoli cells and between Sertoli and spermatids. Thus the gene expression results suggest that BCA exposure disrupts normal ES function and signaling cascades regulating sperm release. Further studies to examine the dose-responsiveness of these effects on gene expression are underway. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy)

849 DRUG-INDUCED HUMAN HEPATOTOXICITY: PREDICTIVE STRATEGIES.

S. K. Durham1 and J. E. Sanders2. 1Discovery Safety Optimization, Bristol-Myers Squibb, Princeton, NJ and 2Safety Intelligence Evaluation, Aventis, Bridgewater, NJ.

Clinical studies have been the pivotal step of drug development in modern medicine. Despite extensive and thorough evaluation in preclinical species, hepatotoxicity is the most common cause for drug failures during clinical trials. Our inability to adequately predict adverse events in patients following the administration of therapeutic compounds is an industry-wide enigma. The enhancement of our knowledge-based understanding of the adverse responses arising from the peculiarities of genetic diversity will greatly improve the robustness of our prediction. The goal of this symposium is to present new developments in our understanding of human
liver toxicity following the administration of pharmaceuticals. A broad range of fruitful avenues worthy of pursuit includes advances in pharmacogenomics, virtual space, and predictive systems utilizing emerging technology platforms.

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**DRUG-INDUCED LIVER INJURY IN MAN:**

**PHARMACOGENOMICS AND PREDICTIVE SYSTEMS.**

**P. R. Watkins, University of North Carolina, Chapel Hill, NC.**

Most drugs that are recognized as capable of causing severe liver injury in man cause no liver injury at all in most treated patients. That is, only a small subset of treated patients are susceptible to the injury. The basis for this idiosyncrasy is widely believed to be hereditary. As genomic DNA is now routinely collected in drug trials, it is possible to test hypotheses concerning specific gene mutations by searching for these mutations in patients with and without signs of liver toxicity, usually serum alanine aminotransferase [ALT] elevations. A problem with this mutation specific approach is that target genes are rarely known, and it is likely that toxicity reflects mutations in multiple genes simultaneously present in the susceptible individual. An alternate approach is to simultaneously screen the genomic DNA pools for many single nucleotide polymorphisms (SNPs) simultaneously to identify regions of the genome that may contain susceptibility genes. When associations are found, the specific genes and mutations can theoretically be identified. A problem with this approach includes the large number of samples that must be analyzed and the current costs of this analysis. It should also be noted that with many drugs, most patients with ALT elevations in clinical trials will have reversal of the elevations, and hence resolution of the liver injury, even if continued on drug. The “adaptation” may involve multiple mechanisms, including immune tolerance. The subset of patients who develop clinically important liver injury may be those with genetic mutations that prevent them from adapting to the initial injury. Since these patients in clinical trials have treatment discontinued when serum ALT rises significantly, it is not possible to identify the poor adapters. This issue may only be addressable after creation of a nation-wide bank of DNA obtained from patients with severe liver injury due to specific drugs.

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**HEPATOTOXICITY: VIEWS FROM VIRTUAL SPACE.**

**G. M. Pearl, Discovery Safety Optimization, Bristol-Myers Squibb, Hopewell, NJ. Sponsor: S. Durham.**

Currently, hepatotoxicity is the most common safety liability responsible for the attrition of drug candidates in the discovery and development process, including black box warnings issued on marketed pharmaceuticals. Despite the importance of this liability, there are no commercially-available predictive computational models for hepatotoxicity. We have established computational modeling algorithms for other safety liabilities that have had a favorable impact on the drug discovery process, especially when these models are used as a sentinel filter for predicting common drug safety liabilities. We are currently developing a predictive hepatotoxicity model that concentrates on the subset of chemical space related to pharmaceuticals. This model is based upon the various statistical algorithms that utilize a central structure activity relationship (SAR/QSAR) scaffold. The standard statistical toolkit, including recursive partitioning, principle component analysis, and neural networks, are especially useful in the development of these models. This presentation will focus on the global strategies, tactical approaches, and infrastructure requirements necessary to establish a robust virtual model of hepatotoxicity.

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**APPLICATION OF EMERGING TECHNOLOGIES FOR THE IDENTIFICATION OF “FINGERPRINTS” OF HUMAN HEPATOTOXICITY.**

**D. M. Dambach, Discovery Safety Optimization, Bristol-Myers Squibb, Princeton, NJ.**

Hepatotoxicity remains the most common cause for drug failures during clinical trials. This is due, in part, to inherent physiological differences between humans and pre-clinical rodent species leading to inadequate prediction of adverse responses in humans. To address this issue, there is a need to develop robust assays and identify biomarkers which are predictive of human hepatotoxicity. The ideal biomarker should be utilized throughout the discovery and development periods to select the safest drugs. Predictive assays, suitable biomarkers, and the determination of the mechanisms of toxicity for compound classes are the new focus areas within drug safety evaluation. An ever-expanding armamentarium of new technologies is being utilized to help identify macromolecular endpoints as potential surrogates of toxicity. The profiles very useful for evaluation include the transcriptome, the proteome, and the metabolome. We applied transcriptional and proteomic analyses in parallel to compare platform technologies and identify potential biomarkers of PPAR-gamma agonist-induced hepatotoxicity. Comparative analysis of oligo-versus cDNA-based arrays, and surface enhanced laser desorption ionization (SELDI) versus LC/MS protein identification methods proved that the benefits of these platforms were additive. The application of both transcriptional and proteomic analyses expanded the potentially useful endpoints for toxicity biomarker identification.

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**COMPARATIVE DRUG-INDUCED HEPATOTOXICITY EXPRESSION PROFILES IN RODENTS AND HUMANS.**

**J. A. Rineinger, Curagen Corporation, New Haven, CT.**

The ultimate goal of gene expression and toxigenomics analyses is to improve the efficiency of drug development by prioritization of lead compounds. Unfortunately, a continuing limitation is the relevance of gene expression profiles derived from animal models to human toxicity and efficacy. The commercial availability of primary human hepatocytes represents a promising means of bridging this gap. However, the use of human hepatocytes in toxicogenomics studies is challenged by variables associated with human donors such as age, gender, ethnic background, and medical history. To evaluate the reproducibility of gene expression studies with primary human hepatocytes, our laboratories have employed GeneCalling® and RT-Q-PCR to generate and compare gene expression profiles from different human donor cells treated with PPAR-gamma agonists. This presentation will also compare the gene expression profiles of PPAR-gamma agonists in primary human hepatocytes to those observed in primary rat hepatocytes and rat liver in vivo. Primary human hepatocytes were non-responsive to prototypical peroxisome proliferator-modulated genes observed in the rodent models. In addition, human hepatocytes showed reproducibility of response in target genes that suggests a different mechanism of action for the fibrate drug class.

**854**

**FACTORS THAT AFFECT THE IMPACT OF DELIVERED DOSE OF INHALED MEDICINES: AN UPDATE OF THE ISSUES.**

**P. A. Weideman and B. D. Smart, Schering-Plough Corporation, Union, NJ and Schering-Plough Research Institute, Lafayette, NJ.**

There have been rapid advances in the use of the respiratory tract as a route of administration for aerosolized medicines to combat systemic disease via the pulmonary vasculature, or to treat lung specific disorders such as asthma, chronic obstructive pulmonary disease or cystic fibrosis. Inhaled pharmaceuticals offer a painless and reasonably convenient technology for the treatment of several life-threatening diseases, but the consistent daily delivery of the prescribed dose of medication is critically dependent upon physical and physiological factors. Exacting aerosol engineering is needed for metered dose inhalers and dry powder inhalers to generate regulated amounts of medicines of defined particle size distributions to reach targeted sites within the respiratory tract. Physiological factors that greatly influence the quantity of drug reaching these sites are breathing patterns, volumes and flows which vary among individuals with age (pediatric vs adult) and extent of pre-existing disease, which may also significantly alter absorption. Following deposition of the inhaled aerosol at the desired site within the respiratory tract, subsequent retention (for pulmonary disease) and bioavailability (for treatment of systemic disease) of the drug are critical factors in minimizing adverse reactions and obtaining maximum efficacy. This workshop should provide the audience with an understanding of new developments in the technology for the delivery of drugs by the respiratory tract administration, and reviews of updated modeling approaches for deposition of pharmaceuticals and current physiological factors that may affect the deposition and bioavailability of these intentionally biologically active, potent compounds. Finally, clinical aspects of inhaled therapeutic aerosols, including current treatments for various respiratory diseases, side effects (or lack of) of inhaled steroids, receptors and targets for maximum therapeutic effect, and innovative therapies using the respiratory system as the portal of entry, will be discussed.

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**INNOVATIONS IN DEVICES AND ENGINEERED POWDERS FOR THE DELIVERY OF INHALEABLE MEDICATIONS.**

**A. R. Clark, Inhalte Therapeutic Systems Inc., San Carlos, CA. Sponsor: B. Smart.**

The first pressurized metered dose inhalers (pMDIs) and single dose dry powder inhalers were introduced nearly 50 years ago. Their introduction marked a new era in the treatment of respiratory diseases. However, these early devices suffer from relatively poor efficiency and reproducibility, typically delivering only 5 to 20% of the
nominal dose to the lungs. While adequate for an inexpensive molecule with a wide therapeutic index, improvements were needed if the lung was going to fulfill its full potential as both a topical and systemic delivery route. Over the last two decades, improvements in efficiency and compliance have been sought in both device design and particle formulation engineering. Initially “add-on” devices for MDIs and multidose versions of dry powder inhalers were developed. Recently, however, fundamentally new device and formulation technologies have emerged. Active devices, which use an internal energy source to supply the energy for aerosol generation, have been developed by a number of companies. Powder technologies, using novel excipients and/or particle morphology to improve powder properties, have also been developed for use with conventional device technologies. While none of these new technologies have reached the market yet they promise improved delivery efficiency, 60-70% of the nominal dose reaching the lungs, and improved reproducibility and compliance.

856 USING MODELS THAT PREDICT INHALED AEROSOL DEPOSITION.


Physical properties of the aerosol and physiological factors of individual subjects affect the amount of inhaled medicine to reach targeted sites within the respiratory tract. Some, but not all, of these factors are included in existing models that predict deposition of inhaled material. These models broadly fall into four basic categories: empirical models, deterministic-(single or typical)-path models, deterministic-multipath models, and stochastic models. All of the models incorporate to some extent physiological aspects of the population. Deterministic and stochastic models incorporate physical properties of the aerosols and mechanisms of airflow and deposition, while empirical models for the most part do not. All models predict total deposition in the respiratory tract, but only a few multipath models predict lobar deposition or deposition relevant to respiratory tract topography. Stochastic models provide the greatest flexibility in representing the impact of intersubject variability on the deposition of inhaled medicinal aerosols. Software programs for some empirical and deterministic deposition models are available for user calculations on a personal computer. Sufficient information on other empirical and deterministic models is available for user programming, while several deterministic and stochastic models are available only at the sites where they were first created. Since most models are compared against a common inhalation exposure database, the predictions of aerosol deposition are similar for standard subject size and breathing rate but begin to diverge significantly for other physiological states. Because of their availability and continued refinement in the incorporation of computational fluid dynamics, deposition models will play a key role in setting design criteria for the next generation of inhalers.

857 PHYSIOLOGICAL FACTORS AFFECTING INHALED AEROSOL DEPOSITION.

B. Phalen, M. J. Okhaimi and O. R. Moss. 'University of California, Irvine, CA and 'CITT Centers for Research Health Research, Research Triangle Park, NC.

The deposition patterns of inhaled particles show enormous variability both among individuals and for a given individual at different times. Theoretical and empirical models indicate that variations in respiratory tract anatomy and breathing patterns are major contributors to this variability. The primary purpose of this presentation is to discuss how age and gender influence airway anatomy and ventilation and thus affect the deposition of inhaled particles. Postnataally, the respiratory tract undergoes significant remodeling and enlargement for about 15 years. During this period, airway size correlates with body size, and changes in oxygen demand produce changes in respiratory frequency and tidal volume. Such anatomical and ventilatory changes after early development appear to be more closely correlated to body size than age per se. Respiratory tract growth appears to cease when general body growth stops. During normal senescence, the changes in anatomy and ventilation are not very well understood, but include an increase in anatomical dead space. Gender-related differences in airway anatomy are largely related to body size effects, but gender-specific characteristics of the upper airways are known to exist. Clinical measurements of aerosol deposition in children and women are consistent with predictions made by using the existing information on anatomy and ventilation. Information on particle deposition in old age is difficult to find. Even when age, gender, body size and ventilation are known, aerosol deposition patterns are variable. It is apparent that additional details relating to respiratory tract anatomy and ventilation must be explored in order to predict the aerosol deposition pattern for any specific individual.

858 THE EFFECT OF PARTICLE SIZE ON THE RETENTION AND DISPOSITION OF INHALED MEDICATIONS.

B. O. Swartz, Schering-Plough Research Institute, Lafayette, NJ.

The aerodynamic behavior of solid dry particles or liquid droplet aerosols of inhaled medications produced from nebulizers, metered dose inhalers (MDIs) or dry powder inhalers (DPIs) determines their deposition site within the respiratory tract of the patient. However, the deposition site environment, including airway or alveolar lining fluids, and availability and viability of pulmonary macrophages, as well as the particle's size and surface area, determine the subsequent disposition and efficacy of the inhaled aerosolized drug. The effectiveness of the inhaled medication in the control of asthma and chronic obstructive pulmonary disease will depend upon its retention time at the target site, with limited systemic absorption. Here the role of drug carrier materials can be important. Conversely, the rapidly expanding use of the respiratory tract as a route for treatment of systemic diseases such as osteoporosis, diabetes or hepatitis has led to increased interest in the rapid deposition of small aerodynamic size inhaled drug particles deposited in the alveolar lung, comprising 100 square meters of surface in healthy adults, plus intimate contact with the extensive pulmonary vasculature. Recently available data and approaches in predictive modeling of pulmonary retention, dissolution and cell mediated clearance will be examined as they apply to the efficacy of inhaled medications.

859 AEROSOL THERAPY FOR PULMONARY AND SYSTEMIC DISEASE: FROM ASTHMA TO DIABETES AND BEYOND.


The past 20 years has seen important advances in treating pulmonary and systemic disease with aerosols. Aerosols provide needless administration, IV-like onset of action, absent first-pass metabolism and a greatly improved therapeutic index. Asthma and chronic obstructive pulmonary disease (COPD) are syndromes that affect about 30,000,000 Americans for which inhaled bronchodilators (beta-agonists, inhaled steroids and/or anticholinergics) (mainly for asthma control) are safe, efficacious, and cost effective in adults and children. Administered by metered dose inhalers (MDIs), dry powder inhalers (DPIs) and small volume nebulizers (SVNs) they are likely to remain the mainstay of therapy for many years. Chronic Pseudomonas aeruginosa infections in cystic fibrosis (CF) and non-CF bronchiectasis are treated with high dose inhaled antileukaglycosides (also cephalosporins or cephalosporins) to improve the therapeutic ratio, increase bacterial killing and minimize bacterial resistance. Antibiotics and inhaled rhDNase, mannitol or hypertonic saline mobilize secretions and improve pulmonary function. In immunosuppressed patients, pulmonary fungal infections, particularly with Pneumoposis carinii and Aspergillus fumigatus, are frequently lethal. For chronic prophylaxis, nebulized pentamidine and amphotericin B, respectively, are safe and effective. Aerosol therapy may also be used for systemic disease. Bronchial asthma is an example of a disease in which there is often significant benefit from the delivery of other medications (e.g., morphine for pain in pulmonary edema) achieve rapid IV-like therapy. Macrolides, (e.g., erythromycin) aerosolization absorption are also effectively used to treat respiratory diseases. Scavenger activity of the alveolus has enabled commercial development of aerosolized inhaled insulin. Additional inhalable macromolecules under active development include growth hormone, parathormone, antiproteases, leuprolide, interferons, and many others. Future inhalables will probably include viral and bacterial vaccines, lung cancer prophylaxis therapy with modified adenoviruses, scavenger activity and radiotracers in addition to IL-2 and GM-CSF. In the coming decade, dozens of important medications will be administered by inhalation and provide needless "therapy without tears".

860 MODELING OF POPULATION VARIABILITY.


One key aspect in toxicological risk assessment is the variability in the population in response to a toxicant. In essence, this variability determines the shape of the dose-response curve (at the population level). Knowledge of the shape of the curve, especially in the lower dose range, is helpful in defining assessment (uncertainty/safety) factors. Such knowledge may reduce the need for such factors, and is helpful to successful risk assessment. Variability may be predicted in pharmacokinetic, pharmacodynamic, and other biomathematical models, e.g., by Monte Carlo techniques, where the output is generated from repeated simulations using parameter distributions, rather than from single simulation with single parameter values. On the other hand, variability of population parameters within a specific model may be estimated by mixed effects modeling or hierarchical population modeling, e.g., in a Bayesian statistical framework using Markov chain Monte Carlo methods.
684 ESTIMATION OF INDIVIDUAL VARIABILITY BY POPULATION PBPK MARKOV-CHAIN MONTE CARLO ANALYSIS.

G. Johnson, Toxicology and Risk Assessment, National Institute for Working Life, Stockholm, Sweden and Medical Sciences - Occupational and Environmental Medicine, University Hospital, Uppsala, Sweden.

The health hazard of a pollutant is in general more closely related to internal exposure to chemical, i.e. target dose, than to external exposure. Thus, population variability in target dose is of great importance in risk assessment. We have used a three-step approach to assess inter-individual variability in target dose and health hazard. In a first step, we retrieve raw data from controlled inhalation exposure of human volunteers to some volatiles (methyl chloride, dichloromethane, toluene, and styrene). We then submit these data to a Bayesian Markov-chain Monte Carlo (MCMC) simulation within a population Physiologically-Based Pharmacokinetic (PBPK) model. The Bayesian analysis makes it possible to merge prior knowledge on model parameters with the toxicokinetic information embedded in the experimental data and yields posterior estimates of the mean and distribution of the model parameters at the individual (representing measurement error and intra-individual variability) as well as the population level (representing inter-individual variability). In a second step, we calculate the population variability in target dose by regular Monte Carlo simulations, combining the results from the first step with population data on age, gender, body build, metabolic genotype, etc., in a population PBPK model. This step has been carried out for methyl chloride (using total amount metabolized at target dose surrogate) and dichloromethane (amount metabolized via the GST pathway). In a third step we calculate the variability in health effect or risk as a function of target dose. So far, we have done this for dichloromethane, by applying the linearized multivariate model on the target dose calculated as above. Obviously, this step requires more quantitative knowledge about the relationship between computational statistic and effect (toxicodynamics) than is presently available for most chemicals. The project was funded by the Swedish council for working life and social research.

685 IMMUNE SUPPRESSION BY TCDD REQUIRES AH RECEPTOR EXPRESSION IN T-LYMPHOCYTES.

L. Kerkeling, L. B. Steppan and D. M. Shepherd, Environ. & Molec. Toxicology, Oregon State University, Corvallis, OR.

The cellular basis for the potent suppression of T-cell-mediated immune responses in mice following exposure to TCDD is not understood. Although activation of the AhR is required, the specific AhR+ cells that transduce the suppression have been difficult to identify in vivo. The recent availability of AhR-/- mutant mice provides novel ways to investigate the direct targets of TCDD. We used an i.p. graft assay to test whether TCDD reduced the ability of T cells to respond to the AhR-dependent effects of TCDD on T cells. In this model, T cells from C57BL/6 (B6) mice were injected into B6D2F1 host mice. The B6 T cells recognize the MHC disparity of the B6D2 host cells, leading to the generation of a CD4+ T cell-dependent CD8+ CTL response that results in loss of body weight by the host. By comparing the ability of TCDD to suppress the GVH response of T cells derived from AhR+/+ and AhR-/- mice, the importance of the AhR in T cells could be determined. We found that the CTL response of T cells from AhR+/+ mice was suppressed when host B6 mice were treated with 15 mg/kg TCDD, and the host mice did not lose body weight. However, when grafted T cells came from AhR-/- mice, there was no suppression of the CTL response by TCDD, and the host animals lost significant body weight. Cells derived from AhR+/- and AhR+/+ mice generated comparable CTL responses in the absence of TCDD, indicating that the absence of the AhR did not alter the innate responsiveness of the T cells. To determine if AhR-mediated effects of TCDD were selective for CD4+ or CD8+ cells, T cells from AhR+/+ and AhR-/- mice were separated into CD4+ and CD8+ subsets, intermixed in various combinations, and injected into host mice. Results showed that the AhR must be expressed in both T cell subsets to have full suppression of the GVH response by TCDD. Future studies will exploit the GVH model using a variety of mutant T cells to directly assess the role of various signaling pathways involved in AhR-mediated T cell dysfunction. (Supported by P01 ES05440 and OSU Unspnsored Research Fund)

686 EXPOSURE TO TCDD SUPPRESSES THE EXPRESSION OF ANTIGEN-SPECIFIC T CELLS DURING INFECTION WITH INFLUENZA VIRUS.

K. A. Mitchell, M. C. Nguyen and B. P. Lawrence, Pharmacology-Toxicology Graduate Program and Department of Pharmaceutical Sciences, Washington State University, Pullman, WA.

One of the most sensitive effects of exposure to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is suppression of the immune system during influenza virus infection. To determine mechanisms by which exposure to
TCDD impairs cell-mediated immunity, we are using a murine model of infection with human influenza A virus. Cell-mediated immunity to influenza virus depends on the activation, proliferation and differentiation of T cells in the mediastinal lymph nodes (MLN). Specifically, activated CD8+ T cells differentiate into cytotoxic T lymphocytes (CTL), which migrate to the lung and kill virus-infected cells. We have previously shown that exposure to TCDD suppresses the generation of CTL in influenza virus-infected mice and this attribute to impaired activation of influenza virus-specific T cells. Thus, we tested the hypothesis that exposure to TCDD impairs the generation of CTL by suppressing proliferation and increasing apoptosis of influenza virus-specific T cells. To follow influenza virus-specific T cells, we used either MHC class I-restricted tetramers or chimeric antibodies specific for the T cell receptor of influenza virus-specific T cells. For these studies, mice were gavaged with TCDD (10 μg/kg) by oral gavage one day prior to i.n. infection with influenza virus. Bromo-deoxyuridine was added to the drinking water (0.8 mg/ml) to examine proliferation. Mice were killed 3 to 9 days post infection and MLN cells were stained and analysed using flow cytometry. Exposure to TCDD caused a three-fold reduction in influenza virus-specific CD8+ T cells that were BrdU+. Contrary to our hypothesis, TCDD had no effect on apoptosis of influenza virus-specific CD8+ T cells based on staining with 7-AAD and annexin V. These findings suggest that decreased cellularity is due to impaired T cell activation, not enhanced cell death. Future experiments will determine whether decreased proliferation is due to the induction of energy in influenza virus-specific T cells.

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EXPOSURE OF MICE TO TCDD DECREASES MUCOSAL IMMUNITY AND INCREASES SUSCEPTIBILITY TO ORAL INFECTIONS BY SALMONELLA TYPHIMURIIUM.
T.R. Jerrells and D. Hoerman. Pathology and Microbiology, University of Nebraska Medical Center and Omaha VA Med. Center, Omaha, NE.

Study results from several laboratories have shown that 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent immunosuppressive compound. Although antibody production is affected by TCDD, the cellular immune system is the most sensitive to the immunotoxic effects of TCDD. It is clear that the systemic immune organs are affected by TCDD; however, the effects of TCDD on the mucosal immune system have not been well studied. The studies described in this abstract were designed to define the effects of TCDD on mucosal immune responses to the pathogenic enteric bacterium Salmonella typhimurium and to relate these changes to changes in susceptibility of mice to oral infection with this organism. It was found that treatment of C57Bl/6 mice with an oral TCDD dose of 13 μg/kg resulted in a significant increase in susceptibility to an oral infection as measured by bacterial numbers in the liver, as well as liver damage determined with histological procedures and serum levels of ALT. TCDD treatment also was found to inhibit the proliferation of mesenteric lymph node lymphocytes to bacterial antigen. To further define the effects of TCDD, mice treated as above were infected with an attenuated strain of S. typhimurium that expresses tetanus toxoid (strain BND 487). The TCDD-treated mice showed a more severe liver infection and pathologic effects by the attenuated bacteria, as well as suppression of lymphocyte proliferative responses to the bacterial antigen or tetanus toxoid. Serum levels of antibody to tetanus toxoid of all IgG isotypes were less in the TCDD-treated mice, but the mucosal immune response was not affected by TCDD treatment. These data show that TCDD suppresses mucosal immune responses and T-cell-dependent antibody production to pathogenic and attenuated S. typhimurium.

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SUPPRESSIVE EFFECTS OF 2, 3, 7, 8-TCDD; CHLOROBENZO-DPIXIN ON THE2- DERIVED CYTOKINE PRODUCTION IN PRIMARY IMMUNE REACTION OF MICE.
K. Nohara 1, 3, I. Ito 1, 2, K. Inouye 2, H. Fujimaki 1, 2 and C. Togaya 1, 2. National Institute for Environmental Studies, Tsukuba, Japan. 1CREST, JST, Kawaguchi, Japan and 2JST, Ibaragi, Japan.

TCDD has been reported to suppress antigen-specific antibody production, but the precise mechanism remains to be elucidated. The type 2 helper 1 (Th2) cell-derived cytokines known to be involved in the humoral immune response. In the present study, we investigated whether TCDD affects the Th2-derived cytokine production by splenocytes in the secondary immune response. In the present study, we investigated whether TCDD affects the Th2-derived cytokine production in the primary immune reaction, where these cytokines play a pivotal role in the proliferation of antigen-specific B cells and their differentiation into antibody-producing plasma cells. Female C57Bl/6 mice (6 week-old) were orally administered TCDD (20 μg/kg) or vehicle alone (corn oil 4% nutrition), and simultaneously immunized with ovalbumin (OVA, 100 μg/mouse).alum intraperitoneally. Four days after the treatment, TCDD significantly suppressed the IL-4, IL-5 and IL-6 production by splenocytes re-stimulated with OVA, while it did not alter the cell numbers and populations of T and B cells. The production of IL-4 and IL-6, but not of IL-4, was suppressed by TCDD from the day 4 through day 14. Among them, IL-5 was particularly sensitive to TCDD and significantly suppressed at a low dose of TCDD-exposure (1 μg/kg). To clarify whether Th cells or antigen-presenting cells (APC) are attributable to the suppression of cytokine production by TCDD, T cells and APC were separated from TCDD- and vehicle-treated mice, and subjected to splenocyte reconstitution cultures. We found that TCDD-treated T cells were essential for the suppression of IL-5 production. In conclusion, these results showed that TCDD exposure suppresses the Th2-derived cytokine production in the primary immune response by mainly affecting T cells, and suggested that these effects of TCDD are involved in the impairment of antigen-specific antibody production.

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THE EFFECTS OF TCDD ON GERMINAL CENTER FORMATION IN THE SPLEEN OF C57BL/6 MICE.
K. Inouye 1, T. Ito 2, H. Fujimaki 1, 2, C. Togaya 1, 2 and K. Nohara 2. National Institute for Environmental Studies, Tsukuba, Japan. 1CREST, JST, Kawaguchi, Japan and 2JST, Ibaragi, Japan.

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) is known to suppress the antibody production in humoral immune response. However, the mechanisms by which TCDD causes immunosuppressive effects remain to be defined. After lymphodepletion antigen (Ag) immunization, Ag-activated B cells migrate into lymphoid follicles to form germinal centers (GCs). In GCs, B cells undergo proliferation and selection to differentiate into plasma cells that produce high-affinity antibodies. The aim of this study was to elucidate at which stage TCDD affects B cell development. We therefore investigated the effects of TCDD on GC formation. Female C57Bl/6 mice were orally administered 0 (vehicle) or 20 μg/kg of TCDD and immunized intraperitoneally with 100 μg of alum-precipitated ovalbumin (OVA) (day 0). In ovalum system, OVA-specific IgG1 in plasma was detected from day 7 (14 days after the first injection). A significant increase in plasmacytoid B cells was observed in the spleen, suggesting that B cells underwent class switch recombination. The antibody production of primary response, measured by ELISA, and the increased proportion of GC B cells in spleen was detected from day 7 by flow cytometry, so that mice were sacrificed on days 7, 10 and 14 to determine the time course effects of TCDD. Suppression of antibody production by TCDD treatment was observed on days 10 and 14. TCDD significantly reduced cell number of GC B cells, as defined by B220-positive B-220 (PNA), from day 7 through day 14, indicating that TCDD inhibited GC formation from its early stage. Besides, the suppression of GC formation by TCDD was also histochemically detected by DNA staining. TCDD induced a characteristic staining pattern of follicular dendritic cells, which are involved in GC formation. These results suggest that TCDD suppresses antibody production through preventing B cell proliferation or differentiation in GCs.

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EARLY T CELL CYCLE BLOCK BY HYDROQUINONE AND CATECHOL.
J. McCave, S. Lutis and B. Pered. School of Medicine, University of Colorado Health Sciences Center, Denver, CO.

Cigarette smoke induces profound suppression of T cell responses in the lung. We have reported that the two major phenolic components of cigarette tar, hydroquinone (HQ) and catechol, block DNA synthesis in proliferating lymphocytes by reversibly inhibiting ribonucleotide reductase. However, inhibition of DNA synthesis following HQ- and catechol-exposure of resting lymphocytes was not reversible, implicating additional mechanisms of inhibition. The purpose of these experiments was to investigate the molecular basis of HQ- and catechol-induced cell cycle arrest. Peripheral blood mononuclear cells (PBMC) were activated with antidi3 and PMA in the presence or absence of 50 μM HQ or 50 μM catechol, and a set of key events that mediate progression through G1- and G2-phases were analyzed. HQ and catechol blocked cells in the S/G2-M phase and also inhibited blast transformation. However, induction of CD25 and CD69 on the cell surface was unaffected, and the production of IL-2 was reduced by only 35-40%, suggesting that HQ- and catechol-treated T cells initiate the activation process, but inhibition of one or more pathways halts further progression. RNA synthesis, which is characteristic of G2-phase entry and progression, was inhibited by greater than 75% in HQ- and catechol-treated cells. Additionally, HQ and catechol diminished the number of transferrin receptors (TR) per cell, and western blot analyses revealed that E2I1-independent gene expression, including genes encoding Cdkl and Mecn1, was reduced by 60-80%. Finally, HQ or catechol exposure prevented dissociation of p24 from p30, which may explain reduced E2F activity. The relationship between inhibition of E2F activity and TR expression is not yet known, but the combined effects clearly contribute to the failure of activated T cells to progress through G2-phase of the cell cycle. To our knowledge, these studies provide the first explanation of how these bencene derivatives affect the first stage of proliferation in any cell type. Supported by the Cancer League of Colorado and NEHS grant ES05673.
EVIDENCE FOR THE INDUCTION OF APOPTOSIS IN IMMUNE CELLS BY DELTA-9-
TETRAHYDROCANNABINOL

R. J. McKelvie, C. Lombard, B. R. Martin, M. Nagarkatti and J. S. Nagarkatti. Microbiology and Immunology and Pharmacology and Toxicology, MCV/VCU, Richmond, VA.

Delta 9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana, has been shown to suppress the immune response. However, the exact mechanism of THC-induced immunosuppression remains unclear. In the current study, we tested the hypothesis that THC impairs the production and effectiveness of proapoptotic cytokines in lymphocyte populations. Splenocytes cultured in the presence of 10 µM or greater concentrations of THC showed significantly reduced proliferative responses to mitogens, including anti-CD3 mAbs, Con A, and LPS in vitro. Thymocytes as well as naive and activated splenocytes exposed to 10 µM or 200 µM THC showed significantly increased apoptosis. THC-induced apoptosis was mediated by the FasL/Fas system, since exposure to 10 µg/ml of THC increased i.p. led to thymic and splenic atrophy as early as 6 hours after treatment. THC-exposure led to reductions in all subpopulation of splenocytes and thymocytes examined. Functional studies revealed that splenocytes from THC-treated mice had significantly reduced proliferative responses to anti-CD3 mAbs. Con A, and LPS in vitro. Induction of apoptosis in the thymus spleen revealed that exposure to THC resulted in increased levels of apoptotic cells in both thymic and splenic cultures. These results suggest that exposure to THC can lead to significant suppression of the immune response; however, the exact mechanism of suppression is under investigation. (Supported in part by NIH grants: RO1ES05958 and RO1HL58641).

EFFECTS OF ETHANOL ON CYTOKINE INDUCTION BY POLYINOSINIC POLYCITIDYLIC ACID (POLY I:C)


Ethanol suppresses Natural Killer (NK) cell activation by poly I:C in a binge drinking model in mice. This leads to diminished resistance to B16F10 tumor cells. Possible mechanisms for this effect are under investigation. In the present study, the effect of ethanol (EtOH) on the induction of cytokines that are important in activation of NK cells was investigated. Mice were treated with poly I:C (0.1 mg/mouse, ip) and EtOH (6 g/kg as a 32% solution in water, by gavage) or corticosterone (18 mg/kg in 2% beta-cyclodextrin, sc) at the same time. Mice were then bled and the spleen was obtained for analysis. RNAase protection assays were used to examine the expression of cytokine mRNAs in the spleen 2 hr after dosing (using a custom probe kit from Pharmingen). Ethanol suppressed expression of IFN-α, IFN-β, IFN-γ, IL-12, and IFN-γ. IL-6 was not affected and expression of IL-10 was increased. Because EtOH induces a stress response, corticosterone was evaluated using a dosing scheme that produces the same area under the corticosterone concentration vs. time curve as measured in EtOH-treated mice. Corticosterone decreased expression of IFN-α, IL-6, and IL-12 but had no effect on the other cytokines. To confirm that changes in mRNA changed the concentration of cytokine proteins, IFN-α, IL-12, and IL-10 were evaluated by ELISA (using kits from Pierce, Biomedical and Pharmingen). All of these three cytokines reached peak levels 4-8 hr after poly I:C. EtOH and corticosterone decreased serum IFN-α from ~300 pg/ml to less than 50 pg/ml at 2 and 4 hr. In contrast, EtOH increased IL-10 concentration, whereas corticosterone had no effect. The levels of IL-12 were significantly decreased from ~300,000 pg/ml to less than 500 pg/ml. These results indicate suppression of NK cell activating cytokines and enhancement of immunosuppressive IL-10 may contribute to the deleterious effect of EtOH on resistance to tumor cells and that corticosterone is not the major mediator of most of these changes. This work was supported by NIAAA grant AA05505.

GALLIUM ARSENIDE INDUCES MACROPHAGE ACTIVATION.

K. L. McCay, M. T. Harrison, S. M. Becker and C. B. Hartmann, Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA.

Gallium arsenide (GaAs), a semiconductor utilized in the electronics and computer industries, has the paradoxical effects of causing systemic immunosuppression and local inflammatory reaction at the exposure site. Macrophages at the exposure site appear activated. To distinguish between GaAs' primary mode of action and secondary consequences, macrophages isolated from unexposed mice and macrophage cell lines were exposed to GaAs in vitro. In culture, GaAs upregulated expression of invariant chain, and the proteins, cathepsin B and L, regardless of the cell source. Invariant chain cleavage was also enhanced. Hence, GaAs directly influences macrophages. However, not all phenotypic changes exhibited by macrophages chemically exposed in vivo were observed after in vitro exposure, such as increased major histocompatibility complex class II expression. These findings raise the possibility that the complete impact of GaAs on macrophages may require cytokine secretion. To study the role of cytokines in GaAs immunomodulation, DNA microarray analysis and mice having disrupted cytokine genes are being used. The ability of GaAs to activate macrophages may contribute to the chemical-induced inflammation at the exposure site. This work was supported by the NIHES grant ES07199, and M. T. Harrison was supported, in part, by training grant T32 ES07087.

CISPLATIN INDUCED GENE EXPRESSION AND ALTERED MORPHOLOGY IN RAT AND HUMAN KIDNEY SLICES.


To improve the prediction for man, gene expression technology in combination with measurements of cell function and morphology has been applied to rat and human kidney slices and to a rat in vivo study, exposed to the broadly used anti-cancer agent cisplatin. Kidney of rats administered 1 mg/kg for 5 days or a single dose of 8 mg/kg exhibited tubular degenerative and regenerative changes, granular and proteinaceous casts and tubular intratubular mineralisation at 96 hours. Kidney slices of rats (10-80 µM) and human (40-500 µM) revealed changes consistent with acute nephrosis of the tubular epithelium. In the rat the cellular injury was localised preferentially in the medulla and at the cortico-medullary junction, whereas in the human kidney slices there was no preferential location. Convoluted regions of the tubules were more susceptible to injury followed by the straight and collecting ductules. Immunohistochemistry staining for apoptosis correlated with the location of damage. In both species, the glomeruli appeared resistant even at high concentrations of cisplatin. The gene categories induced by cisplatin were time and dose dependent and preceded a change in morphology in both species. Gene categories included: apoptosis, DNA damage, cell stress, protein damage, cell cycle/proliferation, metabolism and transport-related genes. In summary, cisplatin-induced nephrotoxicity was demonstrated in rat and human kidney slices by changes in gene expression profiles followed by morphological features. Refinement of the understanding of the gene pathways to better define the mechanisms of toxicity and relate them to interspecies differences in morphology is ongoing. 

CISPLATIN-INDUCED RENAL CELL APOPTOSIS IS p53 AND CASPASE-DEPENDENT AND INDEPENDENT.

B. S. Cummins and R. G. Schindelman. Pharmaceutical Sciences, Med. University of South Carolina, Charleston, SC.

The chemotherapeutic cisplatin causes renal dysfunction and renal proximal tubular cell (RPTC) apoptosis by mechanisms known to involve the activation of caspase 3. The goal of these studies was to determine the mechanism of cisplatin-induced caspase 3 activation and apoptosis. Cisplatin (50 µM) produced time-dependent apoptosis over 24 hr in primary cultures of both RPTC, causing cell shrinkage, a 50-fold increase in caspase 3 activity, a 4-fold increase in phosphatidylserine externalisation, and 5- and 15-fold increases in chromatin condensation and DNA hypophosphorylation, respectively. Caspase 8 and 9 activities did not increase in any time among the 24 hr cisplatin exposure, suggesting these enzymes did not mediate cisplatin-induced caspase 3 activation. Immunocytochemistry and immunohistochemistry analysis revealed that cisplatin treatment of RPTC increased nuclear p53 expression as early as 4 hr after treatment. This increase preceded caspase 3 activation and chromatin condensation by 4 hr. Pretreatment with p53 inhibitor (2-(2-(imidazoo-4-yl)-5, 6, 7-tetrahydrobenzimidazol-3-yl)-1-polyethylene) (PTx, 10 µM) prior to cisplatin treatment inhibited p53 nuclear expression at 4 and 12 hr and totally inhibited phosphatidylserine externalisation and caspase 3 activation at 12 hr. In contrast, PTx only partially inhibited caspase 3 activation, chromatin condensation and DNA hypophosphorylation after 24 hrs of exposure. Neither the caspase 3 inhibitor DEVD-fmk nor the general caspase inhibitor ZAVAD-fmk (50 µM) inhibited cisplatin-induced p53 nuclear expression. Both DEVD-fmk and ZAVAD-fmk completely inhibited caspase 3 activity but, like PTx, only partially inhibited cisplatin-induced chromatin condensation, annexin V labelling and DNA hypophosphorylation after 24 hr. These data demonstrate that approximately 50% of cisplatin-induced apoptosis is dependent on p53 and caspase 3 while another 50% occurs independently of both proteins.

DISRUPTION OF N- AND KSP-CADHERIN LOCALIZATION AND EXPRESSION IS ASSOCIATED WITH MERCURY-INDUCED ACUTE RENAL FAILURE.

L. Jiang, B. C. Burghardt* and A. R. Parish. Medical Pharmacology & Toxicology, College of Medicine, Texas A&M University System Health Science Center, College Station, TX and Veterinary Anatomy and Public Health, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Adhesion molecules play a critical role in maintaining the integrity and polarity of proximal tubular epithelial cells. Although a disruption of cell-cell interactions is suggested in acute renal failure (ARF), little information is known about the expression or integrity of both zonula occludens (tight junctions) and zonula adherens (cadherin) during ARE. The purpose of this study was to investigate the expression and localization of adhesion molecules during mercury chloride-induced
ARF. Male C3H mice were challenged with 25 μM/kg mercury i.p. Plasma creatinine and blood urea nitrogen were significantly elevated at 12, 24, and 48 hr, indicating a reduction of renal function. Western blot analysis revealed no major changes in the expression of tight junction components, ZO-1, ZO-2, or claudin-1 at 48 hr. In addition, no loss of E- or N-cadherin, or β-catenin was seen. However, large decreases in N- and Ksp-cadherin, and α- and p120 catenin were observed. These decreases were not observed at 6 and 12 hr, but were seen following 24 hr of mercury challenge. Immunohistochemistry demonstrated that the Ksp-cadherin was localized along the lumen of renal proximal and distal tubules. At 48 hr, there was a distinct loss of immunoreactivity, and a loss of luminal localization. Similar findings were seen with N-cadherin. Our results indicated that Hg-induced nephrotoxicity is associated with distinct changes in the distribution and expression of zona occludens proteins, most notably Ksp- and N-cadherin, in the absence of alterations in the expression of zona occludens constituents. These results further our understanding of toxicant-induced renal failure, and the potential role of adhesion molecules as molecular targets of xenobiotics.

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SELECTIVE LOSS OF N-CADHERIN EXPRESSION IN THE KIDNEYS OF Aged FISHER 344 RATS, BUT NOT SHR RATS.

S. Gayler, J. Liang, R. C. Burschardt, W. H. Griffin and A. R. Parish.1 Medical Pharmacology & Toxicology, College of Medicine, Texas A&M University System Health Science Center, College Station, TX and Veterinary Anatomy and Public Health, College of Veterinary Medicine, Texas A&M University College Station, TX.

Aging is associated with a loss of renal reserve, and increased sensitivity to either xenobiotic or physiologic insult. Given the critical role of adhesion molecules in maintaining the integrity and polarity of proximal tubular epithelial cells, it was hypothesized that loss of zona occludens (tight junctions), zona adherens (cadherin), or desmosomal proteins may be associated with aging. The purpose of this study was to investigate the expression and localization of adhesion molecules in young (<3 month) and old (24-26 month) male Fisher 344 rats. Western blot analysis revealed no difference in the expression of several components of tight junctions, including occludin, claudin-1, ZO-1 or ZO-2. Similarly, no differences in the expression of desmocollin, desmoglein, or desmplakin, constituents of desmosomal junctions, were observed. However, a pronounced loss of N-cadherin was observed, in the absence of major changes in the expression of E- or Ksp-cadherin. The decreased protein expression of N-cadherin could not be accounted for solely by the loss of proximal tubular cells since Ksp-cadherin is expressed in an overlapping population of cells. As N-cadherin is also present in neurons projecting to the kidney, immunohistochemical analysis was used to demonstrate that the loss of N-cadherin was localized to proximal tubular epithelium. Decreased protein expression of two cadherins, α- and β- was also observed in the aged kidneys with only slight changes seen with γ- or p120 catenin expression. Taken together, these data suggest that aging is associated with decreased expression of N-cadherin and α- or β-catenin, in the absence of significant changes in other adhesion molecules.

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ADVANCING AGE AND GLOMERULAR LOCATION PLAY SIGNIFICANT ROLES IN THE DEVELOPMENT OF RENAL DISEASE IN LABORATORY DOGS.


It is well documented that the incidence of renal disease, and therefore renal dysfunction, increases with age in many species of mammals. Such alterations in renal structure and function may significantly affect long-term toxicology studies. The purpose of this study was to assess the temporal evolution of glomerulosclerosis, an important renal lesion, in laboratory housed dogs, an important model system in chronic toxicology studies. We histopathologically examined representative sections of dog kidneys, quantified glomerular lesions (using the 0-5 scale of the World Health Organization classification system) and performed of statistical analysis of extent and distribution of such changes. The kidney samples were obtained necropsy, and occasionally biopsy, procedures from a collection of 156 purebred Beagle dogs maintained for their entire lifespan in well-controlled conditions. The lesions were correlated with sex, age, and intrarenal location and affected glomeruli to determine the relationship of each in the development of glomerulosclerosis. All dogs examined had some degree of glomerulosclerosis. In the youngest (up to 2 years of age), this was minimal, but was more advanced by middle age (3-7 years). The condition progressed with further aging and was associated with progressive fibrosis and tubular loss. Location and advancing age were significantly related to the development of glomerulosclerosis such that as age increases, the incidence of glomerulosclerosis increases, with the inner ray and inner cortex demonstrating the highest occurrence (p<0.0001 for both). This study indicates that glomerulosclerosis is progressive over the lifetime in a genetically similar population of laboratory dogs.

Beagle dogs maintained under optimal standard environmental conditions. This information needs to be taken into consideration when conducting chronic toxicological experiments using such animals.

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MERCURY DECREASES TRANSEPITHELIAL RESISTANCE IN MDCK I CELLS. ASSOCIATION WITH ALTERATIONS IN CADHERIN/CATENIN COMPLEXES.

A. R. Parish and J. Liang.1 Medical Pharmacology & Toxicology, College of Medicine, Texas A&M University System Health Science Center, College Station, TX.

The loss of transepithelial resistance (TER) in confluent MDCK I monolayers has been used extensively as an in vitro model to investigate mechanisms associated with disruption of cell-cell adhesion. The current studies were designed to determine if mercury chloride is associated with a reduction of TER in MDCK I cells. Cells were seeded on insert-12-well Transwell100 insert chambers and allowed 8 days in culture the cells were challenged with mercuric chloride (0-25 μM) in HBSS. A dramatic reduction in TER was seen with 25 μM mercury at all time-points examined (15-120 min). A loss of TER was also seen with 12.5 μM mercury at 60 and 120 min, with no effect seen at 6.25 μM. The finding that a more dramatic reduction in TER was seen at 15 min when 25 μM mercury was added to the basolateral compartment versus the apical chamber (29 & 52.3% control, respectively) suggests that the adherens junction (cadherin/catenin complex) may be disrupted prior to changes in the zona occludens (tight junction). This finding is supported by Western blot data that demonstrate a small, but significant, increase in the amount of β- and p120 catenin in the Triton X-100 soluble fraction of MDCK cells at 15 and 60 min following mercury challenge. These data suggest that in vitro mercury challenge is associated with a disruption of cell-cell adhesion, as evidenced by the loss of TER, and suggest that alterations in cadherin/catenin complexes may precede changes in the tight junction following mercury challenge.

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TISSUE REPAIR: A KEY TO SURVIVAL FOLLOWING NEPHROTOXIC CHALLENGE.

Y. S. Vaidya1, K. Shankar1, F. A. Lock1, T. I. Bucci2 and H. M. Mehendale1.

1Toxicology, The University of Louisiana at Monroe, Monroe, LA, 2Central Toxicology Laboratory, Syngenta, Keyworth, United Kingdom and 3Pathology Associates International, NCTR, Jefferson, AR.

Trichloroethylene (TCE), a common industrial degreasing solvent, is produced in the United States at about 130,000 metric tons per year. One of the primary mechanisms hypothesized for TCE-induced renal damage and tumors in humans and rodents is the glutathione-dependent β-lyase-mediated formation of S-(1,2-dichlorovinyl)-L-cysteine (DCVC) metabolite in vivo. Our preliminary studies suggested that a timely and adequate renal tissue repair response may be necessary for survival in the animals from a low or medium toxic dose of DCVC. However, inhibition of tissue repair, known to occur after a high dose of DCVC, leads to animal death by renal failure. The objective of this study was to investigate the effects of modulation of tissue repair on the ultimate outcome of renal toxicity. When cell division and timely tissue repair were blocked in vivo, an ordinarily non-lethal dose of DCVC (30 mg/kg, ip) resulted in 90% mortality suggesting that the absence of cell division is very critical for recovery from renal injury of DCVC. The next strategy was to ‘preplace’ renal tissue repair by two different approaches. First approach was to administer a low dose (15 mg/DCVC/kg, ip) 72 hr prior to administering a normally lethal dose (75 mg/kg, ip). Second approach was to administrate mercuric chloride (6 mg/kg, ip), a well-known nephrotoxic. 96 hr prior to a lethal dose of DCVC. In both the cases augmented and sustained cell division and tissue repair (P-thymidine pulse labeling and PCNA immunohistochemistry) induced by the priming low dose resulted in 100% survival inspite of initial massive renal injury. These findings clearly suggest that tissue repair is the key mechanism for survival following an ordinarily lethal nephrotrophic challenge. These results point to novel post-exposure pharmacotherapeutic avenues for treatment of acute renal failure to change the clinical course from hopeless to one of a positive prognosis.

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CALPAINS MEDIATE PROTEOLYSIS OF PAULLIN, VINCULIN, AND TALIN DURING ACUTE RENAL CELL DEATH.


Calpains, Ca2+ activated neutral cysteine proteases, play a critical role in acute renal cell injury/death. However, their cellular substrates in this process remain poorly defined. Paullin, vinculin, talin, and α-actinin are cytoskeleton-associated proteins that have been reported to be calpain substrates. In the current study were identified 1) if paullin, vinculin, talin and α-actinin are calpain substrates during acute renal cell death, and 2) the effects of the calpain inhibitors PD150606 (a
CA2+-binding site inhibitor or SJA 7029 (a catalytic site inhibitor) on these proteins during cell death. Rabbit renal proximal tubules (RPT) were exposed to antimiycin A (10 μM) or subjected to hypoxia for 30 min. LDH release was used as a marker of cell death. Using immunoblot analysis, rabbit RPT expressed 140-kDa, 117-kDa, vinolin, 210-kDa, and 108-kDa α-actin. Treatment of control RPT tissue with purified α-calpain resulted in decreased paternal forms of vinolin, vinculin, talin, but not α-actin. These results suggest that vinolin, vinculin, and talin, but not α-actin, are in vivo calpain substrates. Antimiycin A exposure and hypoxia decreased cytoskeleton-associated vinculin, talin, and α-actin levels, but α-actin, are in vivo calpain substrates. Antimiycin A exposure and hypoxia decreased cytoskeleton-associated vinculin, talin, and α-actin levels, but α-actin, are in vivo calpain substrates.

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QUANTITATIVE RESPONSE FOR FORMATION OF DNA ADDUCTS IN RAT LIVER BY 2-ACTYLAMINOFLUORENE.
G. M. Williams, M. J. Largopulos, J. D. Duan and A. M. Jeffery. Dept. of Pathology, New York Medical College, Valhalla, NY.

We have developed an initiation/promotion model of rat liver carcinogenesis to quantitatively measure effects of genotoxic carcinogens at low exposures for 12 weeks followed by phenobarbital promotion for 24 weeks to elicit tumor development. In previous studies, we demonstrated non-linearities and no effective levels for three of the critical events in tumorigenesis, including DNA adduct formation, DNA repair, bioactivation, liver cell toxicity and compensatory hepatocellular proliferation and induction of preneoplastic lesions, as well as no-effect levels for promotable neoplasia, for 2-acetylaminofluorene (AAF) (Toxicol. Sciences, 45, 152, 1998) and diethylnitrosamine (Arch. Toxicol., 73, 394, 1999). With AAF at the lowest cumulative exposure (120 μmol/kg bw) over 12 weeks, DNA adduct levels after 8 weeks were significantly different from controls, but no promotable liver neoplasia was found. At the next highest exposure (620 μmol/kg bw), DNA adducts were four-fold higher and weakly promotable neoplasia (1 adenoma) was observed. The purpose of the present study was to assess DNA adduct formation and initiation at similar and lower exposures than those used in the previous study. As before, AAF was administered over 12 weeks for cumulative exposure of 2, 4, 42, 420 (the mid dose from the previous study), or 8400 μmol/kg bw followed by 4-week recovery. Increases occurred in relative liver weights, but no increase in plasma alkaline phosphatase or transaminases was evident. DNA adducts, expressed as % of the highest dose (100%) were 0.8, 2.9 and 21.8% for the other exposures, respectively, and 1.1% for the controls. The controls showed 1 spot, one of which was co-chromatographic with the AAF-DNA adducts thus limiting detection of the latter at the lowest dose. Further efforts are underway to resolve this analysis. We conclude that AAF forms DNA adducts at exposures below those producing even early biological markers of initiation.

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ACCELERATOR MASS SPECTROMETRY: A TOOL FOR TRACING ENVIRONMENTALLY RELEVANT DOSES OF 2H AND 3H-LABELED CARCINOGENS.

Accelerator mass spectrometry (AMS) is a nuclear physics technique for quantifying rare, long-lived isotopes with high sensitivity and precision. Consequently, AMS has facilitated in vivo studies at environmentally relevant exposure levels of carbon-14 and tritium labeled carcinogens. We have performed low-level dosimetry studies in laboratory animals, hence establishing the dose-responses for coherent binding of compounds to DNA and protein at ng/kg body-weight doses, with DNA adduct level detection limits as low as 1 adduct/10^8 nucleotides. More recently, human studies have been conducted in which volunteers have been administered dietary relevant doses of heterocyclic amine food mutagens. Adduct levels were 10-fold higher in humans than in rats administered the equivalent dose. Through these studies we are able to more accurately determine the risk associated with environmental exposure to carcinogens. The work was conducted under the auspices of the US DOE by LLNL (W-7405-ENG-48) and partially supported by NIH (NCRR) and DOD.

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DNA ADDUCT CONFORMATION DEPENDS ON DNA CONFORMATION.
G. H. Jiang, M. Skorupski, B. Van Houweling and J. L. Stice. Pharmacology & Toxicology, University of Louisville, Louisville, KY and NIEHS, Research Triangle Park, NC.

Benzo[a]pyrene is metabolically activated to the highly mutagenic derivative (+)-7,8,9,10-dihydrodiol-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE). BPDE-adducts can be one of two stereoisomers and each can adopt several conformations. BPDE-adducts along the DNA are repaired at different rates suggesting that sequence context influences repair rate. How sequence context alters repair efficiency is unknown. Our hypothesis is that the adduct conformation plays a role in the initiation and induction of DNA adducts. To test the role in the initiation and induction of DNA adducts we used BPDE and BPDE-DNA adduct formation. BPDE-DNA adduct formation has 2-5 fold greater than of adducts formed in linear DNAs. Linearizing supercoiled plasmid DNAs after BPDE-DNA adduct formation did not diminish induction efficiency. Heat denaturation and reannealing of supercoiled plasmids decreased induction efficiency suggesting that DNA conformation of the adducts changed. Others have shown that E. coli UVAB-DNA more efficiently incises (+)- cis-BPDE isomers than (+)-trans-BPDE isomers which were both site-specifically constructed into 50-mer substrates. If B. calodermus UVAB works similarly to E. coli UVAb, our results suggest that supercoiled DNA (+)-cis-BPDE-DNA adducts are predominantly formed, whereas on linear DNA the (+)-trans-BPDE isomer adducts predominate. These results support our hypothesis, and these stereoisomeric lesions can serve as a conformational structural tool not only for exploring the mechanism of DNA damage processing by NER but also to provide insights into the potential impact of lesions on mutagenesis. (Supported by NIH Grant R01-ES04660 and the Commonwealth of Kentucky Research Challenge Trust Fund.)
hr. After a thermal hydrolysis, followed by removal of DNA by a microfiltration, N-isopropyl guanidine was detected by an HPLC analysis. The present results suggest that 2-bromopropane might form a DNA adduct in N position of 3'-deoxyguanosine at a physiological condition.

887 IN VIVO AND IN VITRO ADDUCT FORMATION AND MUTATIONAL SPECTRA OF S-(2-CHLOROETHYL)GLUTATHIONE AND N-ETHYL-N-NITROSUREA IN THE P53 GENE.

J. G. Valadex and P. G. Gschwendner. Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN and *Biotecnologia y Bioingenieria, CINVESTAV-IPN, Mexico City, Mexico.

Mutations on p53 tumor suppressor are common in diverse types of human cancers. It is known that p53 binds to DNA in a sequence-specific manner and activates several genes that induce cell cycle arrest or apoptosis following DNA damage. Most of the mutated p53 genes found in human tumors are deficient in sequence-specific DNA binding. The preferential domain of p53 (encoded by the exons 5 to 8) includes the residues responsible for binding to specific DNA sequences. We employed a yeast functional assay consisting of a double-selection method (Environment Mod. Mutagenesis 35:31-8, 2000) to characterize the nature of N-ethyl-N-nitrosourea (ENU) and S-(2-chloroethyl)glutathione (CLEG)-induced mutations of p53 trans-activating activity, in a physiological environment. Both mutagens produced transitions, mostly on C-rich regions. However, the sequence analysis revealed different patterns of hotspots depending on the mutagen used. With ENU high frequency of mutation was observed at codons 141, 177, 194, 199, and 278; with CLEG high frequency was observed at codons 152, 177, 244, and 279. On the other hand, a ligand-mediated polymerase chain reaction (LM-PCR) assay, using fluorescent-labeled primers, was developed to establish if those hotspots corresponded to the sites where adducts are produced in vivo and in vitro by CLEG. We observed a complex pattern of adduced nucleotides, preferentially on C-rich sequences along both strands in exons 5 to 8 of p53. This profile of adducts did not correspond to the pattern of hotspots observed, indicating that different factors are responsible for mutation fixation inside the cell than those revealed by the LM-PCR analysis.

888 THE SCREENING OF TARGET CANDIDATE REGIONS SUSCEPTIBLE TO MUTATIONS IN THE GENOME OF HAMSTER KIDNEY TUMORS INDUCED BY STILBENE ESTROGEN.

K. P. Singh and D. Roy. Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.

Diethylnitrosobenzene (DES) is a renal carcinogen in Syrian hamsters. However, the mechanism of DES-induced carcinogenesis is not clear. In order to understand the sequential molecular events involved in the DES-induced tumorigenesis, it is important to identify the genomic target candidate regions susceptible to mutations. In this study, we used the Random Amplified Polymorphic DNA (RAPD), a polymerase chain reaction-based fingerprinting assay, to screen the entire genome for mutations in DES-induced hamster kidney tumors. Twelve out of hundred primers used for RAPD amplification revealed 22 amplification products harboring mutation(s) in DES induced hamster kidney tumor genome as compared to the age matched untreated control kidney genome. Further characterization revealed that some of these regions harboring mutations are from the coding regions of the kidney tumor genome. Gene expression analysis by RT-PCR revealed that one of the uncharacterized mutated genes is downregulated by several folds in the kidney tumors as compared to the age matched controls. Findings of this study indicates that RAPD is an efficient method to identify the target candidate regions in the genome that are susceptible to mutations induced by exposure to synthetic estrogen, DES.

889 MECHANISTIC STUDIES OF ACRYLAMIDE INDUCED CARCINOGENICITY IN THE F344 RAT.

J. S. Lafferty, L. M. Kamendulis, M. A. Friedman and J. E. Klaung. Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

Chronic treatment with acrylamide in drinking water increased the incidences of adenal pheochromocytomas, mesotheliomas of the tunica of the testes, and follicular adenomas of the thyroid in male F344 rats. The mechanism(s) for these chronic effects of acrylamide is unresolved. The present studies were conducted to determine whether acrylamide or an acrylamide metabolite altered cell growth in the target tissues in the rat. Specifically, DNA synthesis, mitosis and apoptosis were examined in F344 rats treated with acrylamide (0, 2, or 15mg/kg/day) for 7, 14, or 28 days. Acrylamide treatment produced an increase in DNA synthesis in the target tissues (thyroid, testicular mesothelioma, adrenal medulla) at all doses and time points examined. In contrast, in a non-target tissue (liver), no increase in DNA synthesis was seen. No changes in apoptosis or mitosis were observed in any of the tissues examined. Total and relative incorporation of [3H] thymidine in acrylamide induced changes in [3H] thymidine uptake.

890 INDUCTION OF MICRONUCLEI IN RAT HEPTACTYES FOLLOWING DNA DOUBLE-STRAND BREAKS CAUSED BY FURAN TOXICITY IN VITRO.

Y. Zhang, V. A. Wong, D. J. Abernethy, L. Recio and G. L. Kedderis. CTR Centers for Health Research, Research Triangle Park, NC.

Furan is hepatotoxic and hepatocongénoge in rodents but is not mutagenic. Our laboratory has shown that furan metabolites deplete hepatocyte ATP and activate endonucleases that produce DNA double-strand breaks (dsb). We hypothesize that misrepair of DNA dsb results in mutations involved in furan carcinogenesis. Our objective was to determine whether misrepair of furan-induced DNA dsb leads to cytogenetic events such as micronuclei formation. F344 rat hepatocytes were incubated in suspension with furan (0 to 100 uM) for up to 3 hr and then placed in monolayer culture with EGF (50 ng/ml) for 72 hr. The number of hepatocytes with micronuclei stained with DAPI and the mitotic index were determined by fluorescence microscopy. Flow cytometry analysis of BrdU incorporation was used to evaluate cell cycle effects. The spontaneous micronuclei frequency was 6.0 ± 0.7%. Furan treatment significantly increased the frequency of micronuclei in a concentration- and time-dependent manner but did not affect mitotic indices. The micronuclei frequencies for the 3-hr exposure to furan at concentrations of 2, 10, 30, and 100 uM were 7.0 ± 0.4%, 10.5 ± 2.1%, 13.1 ± 1.7%, and 17.8 ± 1.2%, respectively. Both inhibition of furan biotransformation by the cytochrome P450 inhibitor 1-phenyl-2-thiourea (200 uM) and inhibition of the endonuclease activity by sulfinylacetonic acid (100 uM) prevented micronule induction, indicating a requirement for metabolic activation and the need for endonuclease-mediated DNA damage. The labeling index of hepatocytes treated with furan at 30 uM for 3 hr (61.0 ± 6.2%) was significantly greater than that of control cultures (17.6 ± 3.6%), indicating the induction of DNA synthesis. The results of these studies show that hepatocyte DNA dsb formed by furan-induced activation of endonucleases lead to cytogenetic events such as micronuclei formation. The cytogenetic events coupled with furan-induced cell proliferation in the livers of treated rodents are likely to be involved in the development of hepatocellular carcinomas.

891 TOXICITY AND CARCINOGENICITY OF BENZO(A)PYRENE IN SKIN OF DNA REPAIR-DEFICIENT MICE.


In the present study, we hypothesized that transcription coupled repair (TCR) and global genomic repair (GGR) might modulate BENZO(A)PYRENE (BaP)-induced toxicity and carcinogenicity, respectively, in the skin of XP knockout (KO) mice belonging to subgroups C (XPC) and A (XPA). For this purpose, we treated wild type (WT), and XPC and XPA KO mice topicaly with BaP as a single (20, 50 or 200 nmol [group 1]) or multiple doses (20, 50, 100 or 200 nmol) once-weekly for 3 weeks [group 2] or 200 nmol once-weekly for 6 weeks [group 3]. Levels of BaP or diol epoxide-deoxyguanosine (BPDE-DGua) adducts, hyperplasia and bromodeoxyuridine labeling index (BrDU) were examined in epidermis at different time points. Preliminary results showed that Group 1 mice did not differ in the level and disappearance of DNA adducts from epidermal DNA after single dose of BaP except in XPA KO mice, adducts disappeared more rapidly at the highest dose (200 nmol) tested, which paralleled increased epidermal hyperplasia and L1. In group 2 mice, levels of BPDE-DGua adducts were similar between all backgrounds of mice at 20 nmol dose of BaP. However, at and above 50 nmol of BaP XPC KO mice had higher adduct levels than both WT and XPA KO mice. Also in group 3 mice, BPDE-DGua adduct levels in XPA KO mice were lower than WT and XPC KO mice after multiple doses (200 nmol) of BaP. Notably, the decrease in adduct levels in XPA KO mice (Group 2 & 3) again paralleled increased epidermal hyperplasia and L1 suggesting that the lower levels of BPDE-DGua adducts were due to dilution with newly synthesized DNA. Results from a complete carcinogenesis experiment with several doses of BaP suggest that the hyperplasia and cell proliferation induced by higher doses of BaP in XPA KO mice leads to a promoting stimulus. XPC KO mice appeared to be more sensitive to...
from impaired repair of UV induced photoproducts. We hypothesize that there are polymorphic XPA alleles that do not eliminate UV-photoproduct repair and thus do not cause XP disease. These polymorphic XPA alleles may reduce repair of chemically induced DNA damage and may be associated with increased incidence of chemically induced cancers. The XPA DNA binding domain is encoded by exon 4 and 5. Exon 6 encodes the transcription factor (IUH TFIH) binding domain. To identify XPA polymorphisms, PCR amplified fragments containing exons 4, 5 or 6 from 180 random pathology specimens were sequenced. High quality sequence was obtained from fragments containing exons 4 and 6 from 120, exon 5 from 146 samples, and exon 6 from 103 samples. Four SNPs and one single nucleotide deletion were found in exon 4. No polymorphisms were found in exons 4 and 5. Only one exon 4 SNP (T to G at -154 bp 5' of exon 5) creates an alternative splice site. Three single nucleotide polymorphisms (SNPs) were found in exon 6, all 3 of which cause missense changes (Arg238Leu, Val234Leu, Leu253Val). The Arg238Leu and Val234Leu alleles are being tested for functional effects on nucleotide repair capacity. Survival assays suggest that Arg238Leu and Val234Leu have equivalent survival to the wild type allele for both UV survival and benzo[a]pyrene-7,8-dihydriodiol-9,10-epoxide (BPDE) survival. Gene specific repair in the DHFR gene shows that the polymorphic XPA alleles have repair capacity for UV photoproducts equivalent to wild type XPA. Gene specific repair of BPDE-induced DNA damage is currently being performed. Supported by NIH grants ES06640 and RR11803 and funds from the University of Louisville, Kentucky EPSCoR, Markey Cancer Center and Commonwealth of Kentucky Research Challenge Fund.

985 CARCINOGENIC STUDIES WITH ARSENICAL SPECIES IN FEMALE TG.AC (C-GLOBULIN-PROMOTED V-HA-RAS) TRANSGENIC MICE.

986 DICHLOOROACETIC ACID (DCA) TOXICITY STUDY USING Tg.AC AND p53 TRANSGENIC MICE.

987 IDENTIFICATION AND FUNCTIONAL STUDY OF POLYMORPHIC HUMAN XPA GENES.

988 SPONTANEOUS AND DNA DAMAGE INDUCED HOMOLOGOUS RECOMBINATION IN MICE DEFICIENT FOR ATM OR P53.

989 ARSENIC CCARCINOGENESIS: A NEW PARADIGM.
987 SODIUM BROMATE TOXICITY STUDY USING Tg.AC AND P53 TRANSGENIC MICE.

M. Vallant1, J. D. Johnson5, S. W. Graves4, M. R. Ryan1, J. D. Toft, II1, M. Heitman2, G. Boorman1 and R. Chabula1. 1Toxicology, Battelle, Columbus, OH and 2National Toxicology Program, NIEHS, Research Triangle Park, NC.

Sodium bromate, a disinfection by-product (DBP) of drinking water chlorination/ozonation, was administered to Tg.AC and p53 transgenic mice to determine whether these transgenic strains could serve as models to study the carcinogenic potential of DBP chemicals. In addition, the models may serve to rank families of DBP chemicals for toxicity. Tg.AC and/or p53 mice were exposed to sodium bromate in drinking water (0, 60, 400 or 800 mg/L) or by topical application (0, 64, 128, or 256 mg/Kg/day) for 26 (Core) or 53 (Special) weeks. No sodium bromate-related neoplastic lesions were observed in any of the groups. In the drinking-water study, Tg.AC and p53 mice exhibited decreased body weight (>10%) and changes in clinical pathology endpoints. Tg.AC mice also exhibited decreased survival and non-neoplastic changes in the thyroid gland, kidneys, pituitary gland, and testes/epididymides. In the dermal study, Tg.AC mice exhibited decreased body weight (>10%), clinical pathology endpoints changes, and non-neoplastic changes at the site of application, as well as in the thyroid gland, kidneys, spleen, liver, and testes. (Supported by Contract No. N01-ES-65406.)

988 BROMODICHLOROMETHANE (BDCM) TOXICITY STUDY USING Tg.AC AND P53 TRANSGENIC MICE.

J. D. Johnson2, S. W. Graves4, G. V. Martin5, M. R. Ryan1, M. Heitman2, G. Boorman1, R. Chabula1 and M. Vallant1. 1Toxicology, Battelle, Columbus, OH and 2National Toxicology Program, NIEHS, Research Triangle Park, NC.

BDCM, a disinfection by-product (DBP) of drinking water chlorination/ozonation, was administered to Tg.AC and p53 transgenic mice to determine whether these transgenic strains could serve as models to identify the carcinogenic potential of DBP chemicals. In addition, the models may serve to rank families of DBP chemicals for toxicity. Tg.AC and/or p53 mice were exposed to BDCM in drinking water (0, 175, 350 or 700 mg/L) or by topical application (0, 64, 128, or 256 mg/Kg/day) for 26 (Core) or 53 (Special) weeks. No BDCM-related neoplastic lesions were observed in any of the groups. In the drinking-water and gavage studies, mice exhibited increased liver weight and hepatocellular vacuolation (Tg.AC/p53 females); decreased liver weight and hepatic cellular vacuolar changes (p53 males); and decreased kidney weight, nephroathy, and tubular dilatation (Tg.AC/p53 males). No site of application or systemic effects were observed for the Tg.AC or p53 mice in the dermal study. (Supported by Contract No. N01-ES-65406.)

999 USE OF C57BL/6 MICE IN CARCINOGENICITY BIOASSAYS.

M. Atilla1, J. Richard, R. Foster2, V. Paynarchde2 and N. Carmichael1. 1CIF, Envirox, France and 2Aventis CropScience, Sophia Antipolis, France.

We present data from control groups of dietary carcinogenicity studies performed with C57BL/6 mice, for a total of 150 male and 150 female animals. The studies had a duration of 78 weeks. On termination of the studies the incidence survival was 92% for males and 90% for females. The most frequent causes of death were malignant lymphoma and generalised amyloidosis. During the study period average body weight gain for males was 12.2 g; and for females was 12.0 g. Average daily food consumption was 5.2 g (males) and 6.2 g (females). All mice were observed at routine evaluation of the animals during the course of the studies. At histopathological examination of all organs, masses and macroscopic abnormalities, common non-neoplastic pathology findings included subcapsular spindle cell proliferation and cedir degeneration in adrenals, vacuolation of brain white matter, necrosis/necroinflammation of the Harderian gland, mononuclear cell aggregation in the renal pelvis, liver and pancreas, hepatic steatosis, histiocytosis of lymph nodes, atrophic changes in the ovaries, uterus and testes and generalised amyloidosis (adrenals, GI tract, heart, thyroid). Commonly observed tumors included malignant lymphomas (13% of males, 15% of females), hepatocellular adenomas (7% of males, 13% of females), and pituitary adenomas (8% of females). Remarkably few neoplastic lesions were found in the ovaries and testes, especially the lung and the male and female reproductive organs. On the basis of the low and well-defined incidence of non-neoplastic and neoplastic lesions, it is concluded that the C57BL/6 mouse is a suitable model for carcinogenicity testing and that the present data will provide a useful basis for the future use of this strain in our laboratory.

900 COMPARISON OF THE INCIDENCE AND NATURE OF TUMORS IN AD LIBITUM AND RESTRICTED FEEDING REGIMENS FOR CHRONIC STUDIES USING THE SPRAGUE-DAWLEY RAT.


Over the past years investigators working on life span calorie restriction aging and/or carcinogenicity studies have provided unequivocal evidence that the calorie restriction (about 25 to 40% of maximal calorie intake) supplemented with essential nutrients, results in the onset of aging and disease in rodents. Following these and similar findings there has been a move away from ad libitum feeding to restricted feeding in an attempt to increase animal survival by improving the overall health status of the animals. Previous analysis of the data collected at CTBR has shown that animals on these studies had improved survival and had a lower body weight at the end of the dosing period. Lesion data from control animals from carcinogenicity studies using Sprague-Dawley rats from Charles River Canada were compared between restricted and ad libitum feeding regimens. The percentage incidences (total number of masses/total number of rats) of the major type of tumors/lesions were compared for evidence of any significant differences between the two feeding regimens and to determine any populations of tumors/lesions that were unique or predominantly expressed in one of the two feeding regimens. Analysis of the data showed an increased incidence of spontaneous and oncogenic tumors for ad libitum feeding compared to restricted feeding (eg. pituitary adenomas); some significant differences in the incidences between the sexes of certain lesions within the same feeding regimen (eg. mammary gland acacia); and that it was possible to identify tumors that were either unique or predominant to one of the two types of feeding regimens (eg. pituitary adenoma). In conclusion, restricted feeding results in a decreased incidence of spontaneous and endocrine tumors when compared to ad libitum feeding. Thus providing a more sensitive model for determining carcinogenic potential of a test compound.

901 THE EFFECT OF DIET AND ESTRADIOL ON AIC RAT MAMMARY CARCINOGENESIS.


The AIC rat, an inbred cross of Copenhagen Irish and August strains, is a valuable model in the study of factors modulating susceptibility to mammary carcinogenesis. AIC rats are highly susceptible to mammary carcinogenesis when estrogen levels are chronically elevated. This study investigates the effect of a Western-style diet (high in fat and low in vitamin D and calcium) on spontaneous and estradiol-induced mammary oncogenesis. Adult female, ovary-intact AIC rats were implanted sc with control or 20 mg cholesterol pellet or a cholesterol pellet that also contained 1 or 3 mg of estradiol. Rats from each group were fed either the test Western-style diet or the control semisynthetic AIN-76A diet. After 10 weeks of study, one group of rats was sacrificed. There was a significant (p < 0.05) increase in liver wet weight for the estradiol-treated rats compared with the cholesterol-implanted control rats, though not between either of the two doses of estradiol or between the two diets alone. Estradiol caused a significant dose-dependent increase in pituitary wet weight that was not influenced by the diet. A second group was maintained until there was an appreciable mammary tumor burden of the rat became moribund. Rats implanted with the 3 mg estradiol pellet developed palpable mammary tumors at a median of 22 weeks. The diet did not influence estradiol-induced onset of palpable mammary tumors. Histological evaluation of tumors is being evaluated. Rats implanted with 1 mg estradiol and cholesterol are still under study. At this point in the study (32 weeks) the Western-style diet has little or no effect on the development of estradiol-induced mammary oncogenesis in this model. (Supported by ES05022, ES07148, GM49982.)

902 EVALUATION OF THE CARCINOGENICITY OF BISPHENOL A TO RODENTS AND RELEVANCE TO FINDINGS IN HUMANS.

L. A. Highton1, J. J. Huyvaerts1, J. Drolet2, R. Kraaij3, B. S. Lynch1 and J. C. Murphy1. 1Canxas Health Sciences International, Mississauga, ON, Canada, 2University of Kansas Medical Center, Kansas City, KS and 3Utrecht University, Utrecht, Netherlands.

Bisphenol A (BPA) is a monomer component of polycarbonate plastics and epoxy resins, which are used in numerous consumer products, including food-contact plastics. There has been considerable attention and scientific debate regarding the
relevance to humans of reported teratogenic actions of BPA. Conversely, there has been limited consideration of the carcinogenic potential of BPA. The issue of carcino-
genicity is important in the regulation of chemicals for which there is potential for widespread exposure. An evaluation of the carcinogenic potential of BPA to hu-
man was completed using metabolic data, genetic toxicity studies, long-term toxic-
ity/carcinogenicity studies, and estimated consumer exposures. Two-year feeding
studies in rats and mice administered doses containing up to 2,000 and 10,000
ppm BPA, respectively, provided no substantive evidence to indicate that BPA is
carcinogenic to rodents. In addition, a battery of standard in vivo genetic toxicity
assays and a GLP-compliant in vivo mouse micronucleus assay demonstrated a lack of
genotoxic and mutagenic activity of BPA. Metabolism studies showed that ingested
BPA is rapidly metabolized and excreted. Furthermore, it is estimated that cur-
tent uses of BPA, including those of food-contact materials, would result in very
low human exposure. The toxicology data, taken together with the trivial exposure,
support the conclusion that BPA is not a carcinogenic risk to humans.

903 ATRAZINE EXPOSURE IN UTERO INCREASES DIMETHYLBENZ[α]ANTHRACENE-INDUCED MAMMARY TUMOR INCIDENCE IN LONG EVANS OFFSPRING.

S. E. Fenton and C. C. Davis. Reproductive Toxicology Division, USEPA, ORD.
NHEERL, Research Triangle Park, NC.

Recently, we found that ATR exposure during mammary bud outgrowth (late gesta-
tion) delays normal postnatal epithelial progression in Long Evans (LE) to a
greater extent than in Sprague-Dawley (SD) rats. To determine if ATR-induced de-
velopmental delays alter susceptibility of LE females to mammary carcinogenesis, we
gave timed pregnant LE (10 treatment) and SD (11-15 treatment) dams with 0,
12.5, 25, or 50 mg ATR/kg body weight (2×/day) on gestation days 15-19. The
female offspring were gavaged with dimethylnbenz[α]anthracene (50 mg/kg) on post-
natal day 45, a time when mammary glands have largely matured in controls.
Animals were evaluated for palpable tumors weekly and killed 18 and 52 weeks
later, SD offspring exposed to 0, 12.5, 25, and 50 mg ATR/kg (respectively) had a
higher overall mammary tumor incidences of 40, 25, and 52% (X², p<0.15). Their
tumor latency (20, 22, 21, and 22 weeks), multiplicity (2.25, 2.4, 2.9, and 1.5 tu-
mors/tumor-bearing animal), and tumor volumes (3.05, 5.63, 1.81, and 1.08 cm³)
were unchanged by gestational ATR. In contrast, LE offspring exposed to 12.5,
25, and 50 mg ATR/kg had overall mammary tumor incidences of 41, 45, 62, and
68% (X², p<0.014). Although LE displayed no decrease in tumor latency (20.6,
21.6, 18.3, and 21.7 weeks), they had increased multiplicity (1.1, 1.5, 1.7, and 2.0)
and larger tumor volumes (0.2, 0.1, 1.3, and 1.34 cm³, P<0.04) following ATR in
utero. Further, LE (but not SD) rats had significant increases in the percentage of
animals with abnormalities in additional organs compared to controls (i.e., adrenal
nodules, pyloric cysts, ovarian cysts, and lymph node and spleen enlargement)
at all ATR doses tested (P<0.05). Our data demonstrate that by delaying
mammary gland development, gestational ATR exposure increases the susceptibili-
ity of the LE female to carcinogenesis, perhaps by extending the period of vul-
nerability. (This abstract does not necessarily reflect EPA policy.)

904 CHLOROETHANE TOXICITY COMPARED TO STRUCTURALLY-RELATED CHLOROHYDROCARBONS.

J. W. Holder, NCEA/ORD. USEPA, Hazemark, VA.

Chloroethane (CE) exposures at 15 K ppm cause 86% uterine cancer incidence in
B6C3F1 female mice (FM). CE’s most potent carcinogen in the NTP database
by incidence. These cancers are (1) an uncommon tumor type, (2) highly metastat-
ic, and (3) cause of early PM deaths due to tumor load. Contrary to Fish4 rats
similarly exposed were almost tumor free. This analysis examines uptake data into
mice, rats, cats, guinea pigs, and human volunteers and is based on a peer reviewed
a IRIS document and the open literature. Uptakes are similar among species. CE
uptake relates to boiling point, Henry’s constant, blood-air coefficient, and oil dis-
solution. SAR chlorohydrocarbons (CHCs) are compared with CE. Based on the
physicochemical factors, CE shows the least tendency to get in and stay in the body
of any CHC examined. Only high CE levels demonstrate any pharmacologic activity,
analgesic (13 K ppm) & anesthetic (35 K ppm). The lowest recorded chronic
pathologic level (5 K ppm) is where oxidation is saturated and GSH is depleted es-
specially in the adrenals, ovary, uterus, and liver. CE exposures ≥ 5 K ppm may be
less detoxified and more available for toxicity. Hyperkeratotic activity was observed
only in the female mice (FM), but not in MM, MR or FR. Reciprocal running was
observed only during CE exposure which likely produces a state of constant stress
in FM. It’s known human endometrial cancers can be caused by hyperkeratotic ac-
tivity. Thus, the unusual FM cancers may also be based on uterine stimulation.
While uterine cancers are known only at the high CE above, low CE has never
been tested. It’s not likely that lower CE which do not induce stress in FM will
cause uterine carcinogenesis. Re-doing the bioassay to include lower CE exposures,
especially ≤ 5 K ppm, may indicate whether CE is a threshold carcinogen, or not. As
it is, the present data see indicates CE has to considered possibly carcinogenic to hu-
man. Nevertheless, long human use of CE as an anesthetic, with few adverse
reports, indicates the possible carcinogenicity may not hold upon further testing and analysis of CE.

905 ACRYLONITRILE IS A CARCINOGEN IN MALE AND FEMALE B6C3F1 MICE.

B. L. Ghaneyan, A. Nyska and J. R. Bucher. Environmental Toxicology Program,
NIEHS/NIH, Research Triangle Park, NC.

Acrylonitrile is a heavily produced unsaturated nitrile, which is used in the produc-
tion of synthetic fibers, plastics, resins, and rubber. Once considered a probable
human carcinogen, IARC recently changed the classification for acrylonitrile to a
possible human carcinogen. Acrylonitrile is a multi-site carcinogen in rats after ex-
posure to gavage, drinking water, or inhalation. No carcinogenicity studies of acryl-
onitrile in a second animal species were available. The current studies were de-
signed to assess the carcinogenicity of acrylonitrile in B6C3F1 mice of both sexes.
Acrylonitrile was administered by gavage at 0, 2.5, 10 or 20 mg/kg/day, 5 days per
week, for 2 years. A dose-related increase in the incidence of forebrain tubulo-
lar and carcinomas was detected in mice of both sexes with the incidences in the
high dose reaching approximately 60% in both males and females. Higher inci-
dences of focal or multifocal epithelial hyperplasia of the forestomach were ob-
erved in gavage mice. The incidence of hard gland adenomas and carcinomas
also was noted significantly increased in the acrylonitrile-dosed groups. In male mice, the	
incidence of benign or malignant glands in the short term (combined) were
significantly increased in female mice treated with acrylonitrile at 10 mg/kg/day for 2
years. This was also considered an equiva-

906 TERMINAL OBSERVATIONS IN SPRAGUE-DAWLEY RATS AFTER LIFETIME DIETARY EXPOSURE TO POTASSIUM PEFUOROOCANTANESULFONATE.

A. M. Segbat, P. J. Thomford and J. L. Butehroft. 1. Medical, 3M, Saint Paul,
MN and Toxicology, Cansec, Madison, WI.

Sprague-Dawley rats were given potassium perfluorooctanesulfonate (PFOS, 86.7
mg/kg) in the diet at 0, 0.5, 2, 5, & 20 ppm for up to 104 weeks. A recovery group
received compound for 52 weeks. Results from interim sacrifices at weeks 4 and 14
were previously described. An interim sacrifice occurred at week 53 for histopathol-
ogy, cell proliferation and PFOS determination. This report describes observations
at 53 weeks through term. At glucuronidation, lack of cell proliferation and other
cellular changes (withou-

907 TERMINAL OBSERVATIONS IN SPRAGUE-DAWLEY RATS AFTER LIFETIME DIETARY EXPOSURE TO N-ETHYL PERFLUOROOCANTANESULFONAMIDO ETHANOL.

P. J. Thomford, A. M. Segbat and J. L. Butehroft. Toxicology, Cansec, Madison,
WI and Medical, 3M, St. Paul, MN.

Sprague-Dawley rats were given N-ethyl perfluorooctanesulfonamido ethanol (N-
ExFOSE, 98.1%) in the diet at 0, 1, 3, 30, 100 & 300 ppm for up to 104 weeks. A
carcinogenicity study at 100 ppm received compound for 52 weeks. The 300 ppm groups

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were terminated after seven weeks of treatment. Results from interim sacrifices at weeks 4 and 14 were previously described. An interim sacrifice occurred at week 53. This report describes observations at 53 weeks through term. At 53 weeks, decreased body weight increased liver weights and hepatic histomorphologic changes (without cell proliferation), including a hepato celular adenoma in females, occurred at 100 ppm. Through term, survival was unaffected by treatment. Body weights were lower at 100 ppm. All liver effects occurred at 100 ppm, with the exception of cystic degeneration in 30-ppm males. Hepatotoxicity was not present in 100-ppm recipients. In males, thyroid follicular cell adenoma and combined thyroid follicular cell adenoma/carcinoma were increased for trend (p = 0.017 and 0.016, respectively) and pair-wise at 100 ppm (p = 0.011 for both); however, thyroid follicular cell carcinoma alone was not increased. For females, hepato cellular adenoma was increased for trend (p = 0.005) and pair-wise for 100 ppm (p = 0.012) and 100-ppm-recovery (p = 0.050) groups, and combined hepato cellular adenoma/carcinoma was increased for trend (p = 0.002) and pair-wise for 100 ppm (p = 0.007) and 100-ppm-recovery (p = 0.050) groups. There was no increase for trend or pair-wise for hepato cellular carcinoma alone. The only hepato cellular carcinoma observed in the study was in a 100-ppm, terminal-sacrifice female. Significant negative trends and pair-wise decreases at 100 ppm occurred among females for mammary tumors (fibroadenoma, combined fibroadenoma/adeno ma and combined fibroadenoma/adeno carcinoma). Trends for pituitary adenoma and combined adenoma/carcinoma were also negative in females.

908 CARCINOGENICITY OF DEPLETED URANIUM FRAGMENTS IN WISTAR RATS.
E.J. Habib1 and R.A. Guilmette.1, Lovelace Respiratory Research Institute, Albuquerque, NM, and Los Alamos National Laboratory, Los Alamos, NM.

The carcinogenicity of depleted uranium (DU) fragments embedded in muscle was determined in Wistar rats. Tissue reactions to DU fragments were compared to those of foreign-body fragments of tantalum (Ta) and to a radioactive compound, Thorotrast® (Th-90). Flat squares or pellets of the metals were surgically implanted. Three sizes of DU fragments were used, 5×5×1.5 mm, 2.5×2.5×1.5 mm or 2.0×1.0 mm diam. Four fragments were embedded in each rat, 2 in each thigh muscle. Additional groups of rats received Th90 injections in the control for radioactive materials, Ta implants (5×3×5 mm) for controls, for foreign-body materials, or sham surgery as negative controls. Fifty rats per group were observed for life span for the onset, incidence, and types of reactions at the site. At death, all major organ systems were microscopically examined for lesions. Neither type nor amount of implant affected life span. Connective tissue capsules formed around the metal implants, but were much thicker around the DU fragments. Soft tissue tumors arose from some capsules 16 to 20 mo after implantation. Over 90% were malignant. Tumor incidence was significantly increased around the 5×5 mm squares of DU (18%) and the radioactive control, Th90 (50%), when compared with the sham controls (0%). Incidence was slightly increased in rats with 2.5×2.5 mm DU squares (6%) and with 5×5 mm squares of Ta (4%). No tumors were seen in rats with the small DU pellets or in the sham controls. No increase in tumors was found in any other tissues. DU fragments of sufficient size cause local tissue reactions in the muscles of rats that can lead to localized soft tissue sarcomas. Implanting materials into the muscles of rats is sensitive for detecting potentially carcinogenic materials in humans. These findings do not indicate, however, that DU fragments are necessarily carcinogenic in humans. Funding: US Army Medical Research Development Command-MIPR, KVFMS529 with US DOE, Coop. Agreement DHEP-FC04-96AL766. Research conducted using SOT criteria and in AAALAC-accredited facilities.

909 INDUCTION OF LUNG TUMORS BY 7H-BENZO[c]FLUORENE.
1Pharmaceutical Chemistry, Rutgers University, New Brunswick, NJ, 2Department of Environmental Health, Loyola University, Chicago, Illinois, USA.

7H-BENZO[c]FLUORENE (B/cF) is a major lung DNA adduct in mice treated with coal tar suggesting that B/cF may be capable of inducing lung tumors. This study evaluated the tumorigenic potential of B/cF using the A/J mouse model. Female A/J 7 weeks of age were administered BENZ0PIPYRENE, B/cF [10 mg/kg] by ip injection. These mice were fed 5001 rodent LabDiet for the remainder of the study. Groups of mice were also fed diets containing B/cP [357 μmol/kg diet], or B/cP [100 mg/kg diet]. In addition, a mixture of twenty synthetic PAH known to be present in coal tar was also fed to mice in the presence or absence of B/cF. A basal gel diet system was used to administer hydrocarbons within the diet. Mice were maintained on control or adularized diets for 260 days. B/cF administered ip (1.8 mg) induced multiple lung tumors in 92% of the treated mice, with an average of 4.0 tumors per mouse. 90% of the mice treated with 1.8 mg of B/aF by ip administration also had lung tumors with an average of 6.7 tumors per mouse. However, the highest level of lung tumor induction was observed in mice fed 397 μmol/kg diet B/cF. A 100% tumor incidence and an average of 46 lung tumors per mouse was observed. In contrast, mice fed a diet containing 277 μmol/kg B/cP had a 77% tumor incidence with an average of 1.4 tumors per mouse. Mice fed a 27 μmol B/cF per kg diet, or the mixture of 74 synthetic hydrocarbons with or without B/cF resulted in tumor incidences and multiplicities not different from controls. These results demonstrate that B/cF is tumorigenic in lung of mice when administered by ip injection and in particular when fed to mice in the diet. These data strongly suggest that B/cF contributes to the potent mouse lung tumorigenicity previously demonstrated with coal tar when fed to mice. (This research was supported by funds from EPR.)

910 COMBINATION EFFECTS OF HARMAN, NORHARMAN AND AMITROLE WITH NANO2 IN RAT HEPATOCARCINOGENESIS.
T. Ichihara1, K. Miyahita3, M. Kowabe2, K. Imaida1, M. Aasamao1, T. Ogiso1, S. Tamanami1, M. Hirose1 and T. Shirai1.

N-Nitroso compounds, which induce cancers in various organs, may be formed endogenously after intake of amine compounds such as secondary amines and NaN02 in combination. Harman and norharman, EA-carboline derivatives, which are found in some vegetables and in prolyl peptides. A nitrosation had been used in the case. Ethanol, the present study was performed to determine whether typical amines compounds (harman, norharman or amitrole) with NaN02 might have modifying potential on liver carcinogenesis using a medium-term bioassay system. A total of 90 rats were divided into 6 groups, all given a single i.p. injection of DEN (200 mg/kg) at the commencement. After 2 weeks, the rats in group 1 and 6 received basal diet and diet containing amitrole (0.01%), respectively. Animals in groups 2, 3, 4 or 5 received containing basal diet, harman (0.05%), norharman (0.05%) or amitrole (0.01%) combination with 0.1% NaN02 (given in the drinking water) for the remaining experimental duration. All rats were subjected to two-thirds partial hepatectomy at week 3 and survivors were sacrificed under ether anesthesia at week 8 for examination of livers immunohistochemically for glutathione-S-transferase placental form (GST-P)-positive foci. Significant retardation of body weight gain and increment of thyroid weights were observed in rats treated with amitrole. Increase of liver and/or for liver weights were observed in the groups receiving harman or norharman. No modifying potential was evident in terms of numbers or areas of GST-P-positive foci in any groups. These results demonstrate that combinations of harman, norharman and amitrole with NaN02 lack initiating or modifying potential for liver carcinogenesis in our medium-term bioassay system.

911 COMBINED INHALATION AND ORAL EXPOSURES OF MALE RATS TO CHLORFORM ENHANCE CARCINOGENICITY IN THE KIDNEY AND THYROID.

Community residents are usually exposed to hazardous chemicals such as chloroform through multiple routes of exposure in the living environment. For the purpose of assessing health effects of the exposed people, effects of combined inhalation and oral exposures to CHCl3, on carcinogenicity/chronic toxicity were examined in male F344 rats. A group of 50 rats each was exposed through inhalation to 0 (clean air), 25, 50, or 100 ppm (v/v) of CHCl3, vapor containing air for 6 h/day x 5 days/wk x 104 wks, and each inhalation group was given ad libitum either CHCl3-containing drinking water of 1,000 ppm (g) or the vehicle drinking water during the 104-wk period. Incidences of both the renal tumors (renal cell adenomas and carcinomas) and the pre-neoplastic lesional of apical tubule hyperplasia in the kidney as well as incidence of the thyroid tumors (follicular adenoma and adenocarcinoma) increase in the combined exposure groups compared to the groups of the single exposure route. The incidences and/or severities of the non-neoplastic kidney lesions including karyomegaly of the proximal tubule, basophilic change and dilatation of tubular lumens and urinary glomerule were also significantly enhanced in the combined exposure groups, as compared to the groups of the single exposure route. The liver tumors were not observed, but the cases of both liver fatty change and inflammatory cell test were increased primarily in the groups of oral administration. The non-neoplastic lesions of the nasal cavity were observed in the olfactory region, and affected primarily by the inhalation exposure. It can be concluded that the combined inhalation and oral exposures to CHCl3 significantly
enhance the carcinogenicity of the kidney and thyroid in male rats. The non-neoplastic lesions of the liver and kidney were considered to be affected, depending on the oral administration and the inhalation exposure, respectively. This study was contracted and supported by Japanese Ministry of the Environment.

912 PROMOTION OF THYROID FOLLICULAR CELL TUMORS IN RATS BY PREGNENOLONE-16a-carbonitrile (PCN).


Pregnenolone-16a-carbonitrile (PCN) and Aroclor 1254 (PCB) both reduce serum thyroid hormone levels, but only PCN produces an increase in serum thyrotropin (TSH). PCN-mediated increases in TSH result in increased thyroid follicular cell proliferation and hyperplasia, which may represent early events on a morphological continuum leading to neoplasia. The purpose of this study was to assess whether PCN, which induces serum TSH, and PCB, which does not increase TSH, promote thyroid tumors in a 2-stage carcinogenesis model. Male SD rats were administered the thyroid tumor initiator diisopropanolamine (2.5 mg/kg, sc) and after 7 days were fed control diet, diet containing 1000 ppm PCB, or diet containing 100 ppm PCB for 19 weeks. Body weight was unaffected by PCB treatment, however, PCB-treated rats were significantly reduced 21%. PCB treatment significantly reduced serum T4 through week 3 before recovery, whereas T4 levels following PCB treatment fell below detection limits by week 5 and remained drastically reduced through week 19. TSH concentrations in PCB-treated rats increased 3-fold at week 2, then declined to near control values at week 19. Unexpectedly, TSH concentrations increased nearly 2 times control after 1 week of PCB treatment, and were sustained until week 6. The incidence of thyroid follicular cell proliferative lesions, including cystic and follicular hyperplasia, cystic and follicular adenoma, and follicular carcinoma, was significantly increased following PCN treatment but not PCB treatment. PCB treatment caused an increase in the incidence of thyroid carcinomas (4/22 rats) not associated with the proliferative-type lesions produced by PCN, despite an increase in TSH serum concentrations. In conclusion, PCN appears to promote thyroid tumors in a manner consistent with known effects of excessive TSH stimulation. However, thyroid carcinomas stemming from PCB treatment indicate that separate mechanisms exist for the production of thyroid cancer in rodents by chemicals classically considered mitochondrial enzyme inducers. (NIH grants ES-08156 and ES-07079)

913 PROMOTION OF TRIHALOMETHANE-INDUCED COLON ABERRANT CRYPTO FOI (CAF) BY A HIGH-FAT DIET.

M. George, S. Kilburn, T. Moore and T. DeAngelis. National Health and Environmental Effects Research Laboratory, USEPA, Research Triangle Park, NC.

Bromochloromethane (BCM) and dibromochloromethane (DBM) enhanced neoplasia in the large intestine of rats when given by oral gavage; BCM in the drinking water to male rats did not induce colon tumors, but did increase liver tumors. However, TB and a mixture of THMs high in brominated trihalomethanes (THMs) induced colon neoplasia after 52 weeks. We found that brominated THMs induced ACF, preneoplastic proliferative lesions, in the colon when included in the drinking water of male rats. This study was undertaken to determine if the ACF can be promoted by a diet high in fat. Male F344/N rats were exposed to isomolar concentrations of the THMs: 0.5, 0.7, 0.9 and 1.1 g/l of chloroform (TCM), BCM, dibromochloromethane (DBCM) and TB in the drinking water. Emulsified (0.25%) and 15 mg dihydroethaneol (AOM) were the positive control groups. Animals from each treatment group were fed the HF diet (Purina 5001; 19% fat). Groups from the emulsion, AOM, and BCM treatment were given corn oil (0.5 ml/kg, po) 5 days/week. After 26 weeks the colons were examined for CAF. There was a statistically significant positive association between the ACF: (s+SD) and the number of bromine atoms on the THM molecule: 0.25% emulsion (0), TCM (0.07 ± 0.75), BCM (1.33 ± 1.11), DBCM (2.07 ± 2.36) and TB (2.83 ± 2.34). AOM treated animals had 25.17 ± 7.75 ACF/colon. Corn oil did not increase the ACF in the vehicle control or BCM diet treatment groups; but did in the AOM group (32.5 ± 9.0). The HF diet was effective in promoting only the ACF induced by TB (5.33 ± 2.20). The same result was found for the total number of crypts/colon. The data suggest a fundamental difference between THM-induced ACF and those induced by the other THMs, a difference that might help to explain the ability of TB, and not BCM to induce colon neoplasia when administered chronically in the drinking water. (This abstract of a proposed presentation does not necessarily reflect the views of the EPA)

914 THE INDUCTION OF COLORECTAL NEOPLASIA BY A MIXTURE HIGH IN BROMINATED TRIHALOMETHANES (THMS) ADMINISTERED IN THE DRINKING WATER TO MALE F344/N RATS.

C. Sierakowiak, M. George, and T. DeAngelis. USEPA, Research Triangle Park, NC.

The THMs are the most widely distributed and concentrated of the chlorine disinfection by-products (DBPs). Bromochloromethane (BCM) and bromofluoromethane (BMF) administered in corn oil were found to increase the incidence of colon neoplasia in the rat. BCM administered in the drinking water to male rats did not induce colon neoplasia. Since THMs occur together in finished drinking water, we initiated a study to evaluate the ability of a mixture high in brominated THMs (Kraemer et al., J. Amer. Water Works Assoc, 81: 41-53, 1989) to induce colon neoplasia in male F344/N rats. The animals were exposed to mixtures of THMs at 10x, 100x, 1,000x, and 10,000x ambient concentrations. Water containing 0.25% emulsion (vehicle control), 15 mg aminomethanamin (positive control) and 477 mg TBM (to the 10,000X mixture) were included. Colon aberrant crypt foci (CAF); preneoplastic proliferative lesions or neoplasia were scored at 13 weeks. At 13 weeks the incidence of % (animals with ACF) was 100% (AOM, 63% (TBM) and 88% (10,000X THM). The 26 week incidence of ACF was 12% (0.25% emulsion), 100% (AOM and TBM), and 65%, 88%, and 88% in the 100X, 1000X, and 10,000X THM groups respectively. At 52 weeks the incidence of ACF declined in all groups from the earlier time points. Pathologic analysis demonstrated an adenomatous polyp and hyperplasia (17 rats) in the 477 mg TBM/L and adenocarcinoma (17 rats) in the 10,000X mixture treatment group. There are data to support the conclusion that ACF are preneoplastic and colorectal neoplasia is rare in F344/N rats we consider this finding to be biologically significant. This is the first report of DBPs administered in drinking water to test animals inducing neoplasia in animals at a site coincident with the findings of epidemiologic studies. (This is an abstract of a proposed presentation and does not necessarily reflect the view of the EPA)

915 HISTOPATHOLOGICAL EFFECTS OF PROSTAGLANDINS AND ANALOGUES ON THE LIMITING RIDGE OF THE STOMACH IN RODENTS.


The effect of oral exposure of rodents, notably rats, to certain prostaglandins of the E and F series was evaluated, particularly with respect to neoplastic potential. Exposure to misoprostol, 16,16-dimethyl-PGEF, and CL115 574, an older experimental PGE analogue, in the range of 0.1% to 1 mg/kg/day were associated with proliferation of basal epithelial cells of the limiting ridge that separates the gastric stomach from the forestomach. Daily treatment for 10 days to several weeks, depending upon the prostaglandin involved, resulted in gross observation of enlargement of the limiting ridge. Histological sections in animals include hyperplasia, acanthosis, and hyperkeratosis. Eosina may also be present. In addition to specific effects on the limiting ridge, misoprostol, roprostol, natural PGE, 16,16-dimethyl-PGEF, and methyl-PGE, also show trophic effects on the mucosa of the gastric stomach. Prostaglandin-associated pathology of the limiting ridge is non-neoplastic in nature as evidenced by the lack of progression in rat and mouse 2-year studies with misoprostol. Treatment of Sprague-Dawley rat with misoprostol at doses of 0.016 to 0.04 mg/kg/day for 52 weeks produced hyperkeratosis of the forestomach and limiting ridge that resolved following a 13-week recovery period. Reversibility was also shown for 16,16-dimethyl-PGE, and CL115 574. In addition, there is no evidence that prostaglandins, natural or synthetic, possess genotoxic activity. The mechanism(s) by which prostaglandins exert a proliferative effect on the limiting ridge is not clear, but may involve an irritant and a direct trophic effect. Increased cell survival, retarded exfoliation, and the resultant accumulation of cells, may be postulated to potentially contribute to the effects observed. The role of the demonstrated cytoprotective effects of many PGE analogues is not known. Further elucidation of the mechanisms involved may shed light on the relevance to humans of the effects of prostaglandins on the forestomach in rodents.

916 EVIDENCE FOR THE CARCINOGENICITY OF DIAZOMOINOBENZENE BASED ON SHORT-TERM STUDIES.


A series of short-term toxicity and metabolism studies on diazomoinobenzene (DAAB) provide clear evidence that DAAB can be designated as a carcinogen without performing a two-year biosay. DAAB was nominated to the National
The Norwegian National Registry of Severe Allergic Reactions to Food.


Department of Environmental Medicine, NIH-Folkhelse, Oslo, Norway.

The Norwegian Food Control Authority (NTF), Oslo, Norway.

The National Food Control Authority (NTF), Oslo, Norway.

There is a public perception that food allergy is a new concern. However, in some studies, it has been possible to diagnostic verify only 1 out of 5 to 10 self-claimed cases. The prevalence of food allergy in Norway is unknown, and little is known about risk groups, foods, and the circumstances under which the reactions occur. Diagnostic follow-up and patient care appear to vary greatly. Patients often feel that their problems are neglected. In the present study, we have used a newly developed system to gather information on severe allergic reactions to food. The purpose is to obtain data for the incidence and other information about severe allergic reactions to food in Norway, in the first instance as experienced in the health care system patient interface before more effective diagnostic work has been performed. Furthermore, we also want to know the distribution of patients' diseases. Severe food allergy is a frequent and serious problem. The prevalence of food allergy in Norway is unknown, and little is known about risk groups, foods, and the circumstances under which the reactions occur. Diagnostic follow-up and patient care appear to vary greatly. Patients often feel that their problems are neglected.

The Norwegian Food Control Authority (NTF), Oslo, Norway.

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can increase the release/yield of radio-label 5-HT (1H-5-HT) from rat brain cortex (FASB J., 15: Abs. 207-13, 2001), and this could explain HCA's reported role in appetite suppression. Although HCA has been reported to suppress appetite and reduce food intake in experimental animals and humans, its mechanism of action is not fully understood. In the present study, we investigated the possibility that the observed effect of HCA on 1H-5-HT release from isolated rat brain cortex could be mediated by an action on 5-HT uptake. Isolated rat brain cortex slices were initially incubated in oxygenated Krebs solution for 20 minutes and then transferred to buffer solutions containing [1H]-5-HT for different time intervals. In test experiments, tissues were exposed to 1H (10 μM - 1 mM), fluoxetine (100 μM) and clozapamine (10 μM). Uptake of [1H]-5-HT was expressed as d.p.m./mg wet weight. A dose-dependent decrease in 5-HT uptake was observed when [1H]-5-HT occurred in cortical slices reaching a maximum at 60 mins. HCA, fluoxetine and/or clozapamine inhibited the time-dependent uptake of [1H]-5-HT. At 90 mins, HCA (300 μM) caused a 20% decrease whereas fluoxetine plus clozapamine inhibited [1H]-5-HT uptake by 30%. Thus, HCA can inhibit [1H]-5-HT uptake (and increase 5-HT availability) in isolated rat brain cortical slices. A similar effect was also observed byeffect on those of electrophysiological currents evoked by [SSII] and may have the antidepressant properties of fluoxetine. In addition, safety studies including acute oral toxicity, dermatotoxicity, dermal irritation and eye irritation, were conducted to determine HCA's safety profile. The LD50 of HCA was found to be greater than 5000 mg/kg when administered orally, and other results were encouraging. Taken together, these results demonstrate that HCA is safe and helps suppress appetite.

922 BUTYLATED HYDROXYTOLUENE INHIBITS AFGAFFICITY AND RELATED METABOLIZING ENZYMES IN TURKEY LIVER MICROSONES.
J. A. Guarino, P. J. Klein and R. A. Coughlin. Toxicology Graduate Program, Department of Veterinary Science, Utah State University, Logan, UT.

The mycotoxin aflatoxin B, (AFB) is a potent hepatotoxic, and a common contaminant of foods and feeds. Turkeys are extremely susceptible to the toxic effects of aflatoxin. A condition we have shown to be due to a combination of efficient hepatic cytochrome P450 (CYP)-mediated activation and deficient glutathione S-transferase (GST)-mediated detoxification of AFB. We recently demonstrated that butylated hydroxytoluene (BHT) protects against nearly all of clinical signs of aflatoxins in turkeys, an effect that is not mediated by induction of GSTs. The purpose of this study was to determine the protective mechanism of BHT-pretreated poultry against aflatoxicosis in turkeys. Our data show that BHT is a competitive inhibitor (Ki = 5.38 mM) of AFB, activation to the AFB, 8, 9-epoxide (AFB-O) in liver microsomes prepared from male white hens. Furthermore, BHT inhibited microsomal ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-deethylase (MRD), and pentoxyresorufin O-deprenylase (PROD), indicating activities of CYP1A1, 1A2, and 2B1, respectively, in a dose-related manner. These results suggest that BHT inhibition supports an earlier observation of AFB activation in turkey liver is mediated by a CYP 1A1 homolog. It is therefore possible that the observed chemoprotective properties of BHT in turkeys may be due, at least in part, to its ability to inhibit hepatic AFB activation to reactive intermediate(s). (Supported by USDA-NRI grants 97-37081 and 98-3794).

923 CHEMICAL CHARACTERIZATION OF CHROMIUM PICOLINATE MONOHYDRATE.

NTP is conducting rodent 90-day toxicity and 2-year carcinogenicity studies of chromium picolinate, which is a nutritional supplement that is being marketed for weight loss. The present work describes the methods and results of studies to confirm the identity of the bulk chemical and to estimate its purity and stability. IR, 1H-NMR, and XRD spectroscopy were consistent with the structure and identity of the bulk chemical as chromium picolinate monohydrate (Cr(III)H(OH)2). Cr(III)H(OH)2/IC-ESI/MS revealed the presence of an intense ion at m/z 436 consistent with the [M + H]+ ion of Cr (Pic)3·H2O. The presence of one mole of water in the complex was also revealed by Karl Fischer titrometry. Elemental analysis for carbon, hydrogen, nitrogen (98.5%, 95.9%, and 97.3%, respectively, of theoretical values), and ICP-AES analysis for total chromium (133% of the theoretical value) supported the identity of the bulk chemical as Cr(Pic)3·H2O. Total impurities detected by HPLC were <0.5%. Using LC-ICP/MS, the maximum concentrations of either free Cr(III) or Cr(VI) in the bulk chemical were < 0.025%. Purity evaluation using UV detection at 265 nm revealed a major component at -56%, one impurity present at 2.1%, and an additional five impurities each present at levels < 0.7%. The presence of impurities was not unexpected since several chromium/picolinate complexes can arise from the aqueous phase equilibrium of this compound. LC-ESI/MS revealed that many of the components present in synthetic mixtures of chromium/picolinate complexes were also present as impurities in the bulk chemical, but unequivocal identification of the components was not possible due to the lack of pure authentic reference standards. Stability studies revealed that the bulk chemical was stable for at least 28 days at room temperatures up to 60°C, demonstrating that storage at room temperature is acceptable for long-term studies.

924 THE EFFECT OF TRIBUTYLLIN ON MICROBIAL INFECTIONS OF MICE.

Trubutyltin compounds (TBTs) are immuno-toxic chemicals, having been artifically introduced to marine environments. Recently, the contamination of more than 1ppb TBTs has been found in most of fishes yielded in Japanese fish market. This suggests continuous exposure of TBTs to the Japanese people through direct ingestion of fish or other sea foods. In the present study, the effect of tributyltin chloride (TBTCl) by oral exposure was examined on host resistance of ICR strains mice against challenge of microbial infection. Off-spring mice received TBTCl from lactation of mother mice that had taken drinking water with 0, 5, 15 or 50 ppm TBTCl. On the morning of day 21, they were infected with Listeria monocytogenes or Candida albicans. The results of these microbial infections were estimated by the numbers of recovered microbes in spleen at the day 2, 4 and 6 for L. monocytogenes infection, and by those in kidney and brain at the day 43 for C. albicans infection, respectively. The Listeria was detected in spleen throughout the experiment obtained from off-spring groups that were lactated by mother given TBTCl at the concentration of 0, 5, 15 or 50 ppm. Candida was also found in the brain of off-spring groups given 50 ppm TBTCl, while no Candida was detected in the control group without TBTCl. In addition, FACS analysis at the weighing point showed that TBTCl induced the slight decrease of CD8+ T cell population in spleen. These results suggest that the exposure of the extremely low levels as 50 ppm TBT through lactation by mother leads to decline of host resistance against microbial infection in off-spring.

925 YEAST ESTROGEN SCREEN TO TEST FOR ESTROGENIC ACTIVITY OF VARIOUS INFANT FOODS.

High intake of estrogenic compounds has raised concern about potential adverse effects on reproduction and development, especially in infants as susceptible subgroup of the population. Intake estimates are usually based on analytical chemistry and take not into account actual biological activity. Yeast stably transfected with the estrogen receptor and a reporter construct is a commonly used assay to screen for estrogenic activity of chemical compounds. The purpose of our study was to use this screening tool, expressing the human ERa, to test for estrogenic activity in various infant foods. Soy infant formula, containing isolatoves, are compared to milk-based formula, human and cow's milk, which may contain phytoestrogens as well as steroidal estrogens. We screened the extracts of the various foods as well as directly the diluted or reconstituted formulas as the main conjugated and free isolatoves as well as steroidal estrogens. Overall activity of soy formula was low compared to steroidal estrogens and isolatoves. No activity was detectable in any of the foods under the conditions of our test system. Extracts of the foods are easier to analyze; reconstituted milks tested directly influenced the cell growth and interfere with OD measurements. Since the compounds tested in this estrogen screen have different chemical structures with different physico-chemical properties, we tested the penetration of test compounds into the yeast cell, using C14-labelled estradiol and C14-labelled genestin1. In both cases the rate of penetration was below 1% of the total amount added after over night incubation. Although a fairly easy and simple to use system, the yeast estrogen screen allows only for relative comparison of overall estrogenic activity of food extracts and for screening purposes of pure compounds. Conclusions on potential health effects of high intake of dietary estrogens cannot be drawn based on results from such an assay. More information on in vivo uptake, metabolism, distribution and internal exposure in target organs is necessary.

926 ASSESSMENT OF THE ALLERGENICITY OF KNOWN HUMAN ALLERGENIC AND NON-ALLERGENIC FOOD PROTEINS IN MICE.
D. A. Dekker, C. Smith, J. P. Stolkey, R. S. Scott, G. S. Ladds and C. S. VanPelt. Discovery Toxicology, DuPont Nutrition Laboratory for Health and Environmental Sciences, Newark, DE.

No validated animal model exists which can predict protein allergenicity. A model has been proposed by Deardor et al, that has exhibited divergent antibody isotype responses (IgG vs IgG2a) in female BALB/c mice exposed IP to a known human allergen, ovalbumin (OVA) vs a non-allergen, BSA. To examine the reproducibility
and predictability of the proposed model, two additional proteins, potato acid phosphatase (PAP) and ß-lactoglobulin (ß-Lac), and two additional strains of mice, C3H/HeJ and A/J, were evaluated for allergic responses. Ten female mice of each strain were IP injected once a week for two weeks with 1, 2, or 5% BSA, OVA, ß-Lac, or PAP in PBS buffer. One week after the last injection, animals were euthanized and blood was collected from the vena cava. Serum antigen-specific IgG, IgG1, and IgG2a were determined by ELISA and bioactive IgG to each protein was determined by PCA in naive rats. Serum antigen-specific IgG was observed in a dose dependent manner for all four proteins tested. Overall, BSA demonstrated the highest titer of IgG followed by OVA, ß-Lac, and PAP. Unlike the results of the Dearnay study, bioactive IgG was not observed in BALB/c mice treated with OVA, or with ß-Lac or PAP, a human food allergen and non-allergen, respectively. A/J mice treated with 2 and 5% OVA demonstrated clinical signs of anaphylaxis. Bioactive IgG was observed in A/J mice treated with OVA at all dose levels. Bioactive IgG was also observed in A/J and C3H/HeJ mice treated with PAP and ß-Lac. The strongest positive reaction was seen in BALB/c mice injected with PAP. In a panel of responder mice and antibody titer were observed in A/J mice treated with 2% OVA or 3% PAP. Bioactive IgG was not observed in any strain treated with BSA. These results suggest that the production of bioactive IgG is dependent on the genetic background of the mice exposed and the dose of protein that is administered. The IgG response to each protein is more uniform and may be a useful means of determining exposure to a test protein.

927


The National Residue Program (NRP) conducted by the USDA Food Safety and Inspection Service (FSIS) includes a comprehensive testing program for residues of pesticides, drugs, and other chemical contaminants in meat, poultry, and egg products. Samples are taken statistically so that inferences can be made on the overall national incidence of residues. Since 1990, there has been a large decline in the number of horses slaughtered for food in the US. The number of horses slaughtered for food in the US has decreased steadily in the past 15 years. The number of horses slaughtered in 1999 was 266,000. Of this number, 20% of the number slaughtered in 1990 (603,000) was reported in 1999. In 1999, 364,000 samples were analysed in horses between 1990 and 1999. During this period only 31 violations were found, resulting in a violation rate of 0.6%. The violations consisted of the following: five camphor violations; two hepatocarcinoma; and three PCB violations. In addition, FSIS detected 329 non-violative positive residues (i.e., residues present at levels less than or equal to the tolerance or action level). The compounds found at non-violative levels were: dieldrin, DDT, heptachlor, lindane, methoxychlor, heptachloroepoxide, imirex, endosulfan, PCB's, chlorinated phenols, and others. Since 1977, the United States Environmental Protection Agency has banned all uses of DDT, except for public health emergencies. Nevertheless, non-violative residues of DDT were found every year except 1990, with the highest numbers in 1993 and 1998 (40 and 25, respectively). This can be explained by the environmental persistence of DDT residues that were introduced into the environment in the 1972 ban. These findings indicate the value of the FSIS NRP in assessing the exposure to these chemical residues, and in maintaining consumer confidence in the safety of the US food supply by demonstrating that the incidence of violative levels of these contaminants is very low.

928

RELATIONSHIP OF TRICHTOHETEHCENE STRUCTURE TO COX-2 INDUCTION IN THE MACROPHAGE MODEL.

Y. Moon and J. Jin. Dept. Food Science and Human Nutrition, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.

Cyclooxygenase-2 (COX-2) is a crucial rate-limiting enzyme in the production of proinflammatory eicosanoids. Previous studies have shown that deoxyivanol (DON) induced COX-2 expression by promoting transcriptional activation and mRNA stability via nitric-oxid activated protein kinase (MAPK) signaling pathway. Based on this model, representative members of the three major trichothecene families (A, B, and D) were screened for the inducibility of COX-2 expression using murine macrophage, RAW264.7 cells. When treated with the 20% inhibitory doses (ID50) based on MTT assay, Type B trichothecenes including DON, 15-acetylDON, 3-acetylDON and fusarenon-X (FX) were potent inducers of COX-2 expression whereas Type A and D had little effect on COX-2 expression. To assess the effect of the trichothecenes on transcriptional and post-transcriptional activity, luciferase reporter vectors containing 5 promoter or 3 untranslated region (UTR) of COX-2 gene were transfected into RAW 264.7 cells and the reporter activities were compared following the trichothecene treatment. Typically, type B trichothecenes enhanced the COX-2 promoter activity and UTR-linked mRNA stability whereas Type A and D did not affect the reporter activities. The COX-2-inducing trichothecenes also activated MAPK signaling pathways, and ERK or p38 inhibitor significantly reduced the COX-2 induction. In contrast, the dominant negative JNK expression did not diminish the trichothecene-induced COX-2 expression although JNK was activated by the four toxins. Additionally, pyridinidine dithiocarbamate, an inhibitor of nuclear factor-kB (NF-kB) activation, suppressed the trichothecene-induced COX-2 expression. Taken together, type B trichothecenes including DON, its derivatives and FX are direct strong inducers of COX-2 gene and the induction is due to both enhanced transcription and mRNA stabilization. Furthermore, MAPK and NF-kB signaling pathways mediate the trichothecene-induced COX-2 expression, which may contribute to the type B trichothecene immunotoxicity.

929

SAFETY EVALUATION OF EPHEDA DIETARY SUPPLEMENTS.


The safety of ephedra/ephedrine alkaloids has been studied in animals and humans. A series of clinical investigations have been done with these, but otherwise healthy volunteers receiving oral ephedra or ephedrine over periods of 10 to 26 m. The range of total doses within these studies was from 50 to 150 mg/d (the LOAEL) in 1 to 3 doses. Monitoring data for adverse effects identified the NOAEL of 90 mg/d reported symptoms such as chest pain, palpitation, irritability, nausea, and constipation were similar among supplement and placebo groups. Carcinogenity studies in mice and rats demonstrated a lifetime NOAEL of 9 mg ephedrine/kg bw/d. The only finding at this dose was decreased body weight gain, which increased, or had no effect on survival, these lifetime rodent studies showed that there were no long-term, cumulative effects that may not be detected in clinical studies. Using the results obtained from these investigations, application of the National Academy of Sciences Upper Level (UL) Model derived a UL of 90 mg of ephedrine alkaloids in ephedra for a generally healthy population. This assessment included the use of an Uncertainty Factor of 1, applied to the human NOAEL. This UL is based on consideration of the pharmacokinetics of ephedrine, use patterns, duration of expected use, animal and clinical studies, without consideration of the voluntary database of adverse event reports that we found to be generally unreliable and insufficient to draw any conclusion about the safety of ephedra dietary supplements.

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13-WEEK FEEDING STUDY IN RATS FED GRAIN FROM YIELDGARD® EVENT MON 810 CORN.


As part of the safety assessment of feed/food derived from genetically modified crops, it is important to evaluate unintended consequences owing to the insertion of the transgene into the genome or expression of the resulting trait. In combination with other approaches such as compositional evaluations and nutritional assessments in farm animal feeding studies, a subchronic feeding study in rats may address such unintended effects. This study compared the responses of rats fed diets containing grain from Yieldgard (YG) corn to rats fed (1) diets containing grain from near-isogenic control (C) (non-transgenic) corn and (2) populations of the YG grain from commercial reference corn (RC) hybrids. Male and female CD IGS rats (20/sex/group) were fed one of the following diets for 13 weeks: 11 or 33% (wt/wt) (YG) or (C) grain; 33% (RC) grain (6 commercial lines were tested). There were a total of 10 groups/400 rats in the study. Diets containing 11% YG or C grain were supplemented with 22% grain (non-transgenic commercial corn) to bring the total corn grain to 33%, consistent with other diets. Laboratory rat feed contains corn grain at 33%. Diets were balanced to be nutritionally equivalent. Animals were observed daily; body weights and food consumption were recorded at least weekly. At 5 and 13 weeks, blood and urine were collected from 10 rats/sex/group for clinical evaluations. After 13 weeks, all animals were sacrificed, necropsied, and selected tissues weighed. Histologically, Body weights and food consumption were comparable for all groups. Clinical parameters were comparable across groups with few exceptions. The few differences were not considered biologically meaningful as they were either not dose related, or were not consistently different from both control groups. In conclusion, rat fed grain from Yieldgard corn responded similarly to rat fed control grain. This conclusion was consistent with previous studies showing that YG corn grain is compositionally and nutritionally equivalent to traditional corn.
A one year tolerance study was performed in cynomolgus monkeys to evaluate the toxicity of lutein and zeaxanthin administered at 10% beadlet formulations by gavage at 0, 0.2 and 20 mg/kg/day (pure Carto-Kote) to groups of 2 male and 2 female cynomolgus monkeys. Additionally, 1 male and 1 female animal at 20 mg/kg/day in each sex were also sacrificed after 6 months of treatment. All animals survived the 6 months of the study period. There was no observed effect on overall mean body weight, nor on any other parameters assessed, including blood pressure, heart rate, body temperature, and behavioral changes. The study was concluded and lutein and zeaxanthin are safe for use in humans.

Phytochemicals are structurally very similar to cholesterol and their cholesterol-lowering ability has been well documented since the 1950s. Added to vegetable oils, spreads or the fatty acid esters they have been marketed as a cholestrol lowering food. The safety of phytochemicals and their functional activity has been established and has been found to have positive effects on various health indicators.

RESPONSIVENESS OF HEAT SHOCK PROTEIN GENES TO A RANGE OF HERBAL SUPPLEMENTS.

M. Xie and B. Yam. Biomedical Science, University of Rhode Island, Kingston, RI.

The genes that encode heat shock proteins (HSPs) are coordinately regulated in response to acute exposure of cells to a range of physiological and environmental factors. The present study was undertaken to test the activation of the heat shock response by herbal products and herbal extracts in cynomolgus monkeys. The study involved the administration of herbal supplements to groups of 2 male and 2 female cynomolgus monkeys. The supplements were administered orally and included various herbal extracts and botanicals. The study was concluded and the results indicate that the herbal supplements are safe for use in humans.

13-WEEK FEEDING STUDY IN RATS FED GRAIN FROM ROUNDUP READY® @ Venlar NK600 CORN.

B. G. Hammond, B. R. Dudek, M. A. Nemeth and J. D. Axwood. Monsanto Company, St. Louis, MO.

As part of the safety assessment of feed/food derived from genetically modified crops, it is important to evaluate unintended consequences owing to the introduction of new traits. This study compared the responses of rats fed diets containing different concentrations of genetically modified corn from Roundup Ready®. The study involved the administration of different concentrations of genetically modified corn to groups of 2 male and 2 female rats. The results indicate that the genetically modified corn is safe for use in humans.

COMPARATIVE ACUTE AND COMBINATIVE TOXICITY OF AFFLATOXIN B1 AND T-2 TOXIN IN RATS AND FISH.


The study involved the administration of different concentrations of genetically modified corn to groups of 2 male and 2 female rats. The results indicate that the genetically modified corn is safe for use in humans.
in F-344 rats and Mosquitofish ( Gambusia affinis ). Young male rats were randomly divided into five groups and were orally treated with 1.0, 4.64, 2.15, 1.0, and 0.2 mg/kg AFB1 or T-2 according to the horn's method. The rats were observed for 7-appearing rats after 48 h, and death of T-2 treated rats, within 24 h. The LD50 for AFB1 was calculated as 2.71 mg/kg with the 95% confidence limit (CI) at 2.00-5.01 mg/kg for T-2. Healthy fish fed AFB1 were killed at 95% CI at 2.00-5.01 mg/kg for T-2. Healthy fish treated were observed for 5 days and death and toxic signs of fish were recorded. The LC50 for AFB1 was calculated to be 681 ppb with 95% CI at 420-880 ppb and LC50 for T-2 was 1470 ppb. Fish show more sensitivity to the acute toxicity of T-2. Combining studies used both biotoxins at doses of 1.0, 3/4, 1/2, 3/8, 1/4 LD50 LC50 in both species. These results will form the basis for our future study on combinatorial toxic and health effect of these biotoxins and their combinations. This work was financially supported by the research contract DDAD1-3-00-C-005 from Soldier and Biological Chemical Command (SBCCOM), US Army.

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A NEW AETIOLOGY FOR RHABDOMYOSIS: TRICHLOMA SPECIES INTOXICATION.
I. Baedrimont', R. Bedry', G. Deffense', and I. E. Coggan', 'Toxicology, Université de Bordeaux 2, Bordeaux, France, 'Resuscitation and medical toxicology, Université de Bordeaux 2, Pessac, France, and 'Mycology and vegetal biotechnology, Université de Bordeaux 2, Bordeaux, France.

Tricholoma asperellum is a well-known mushroom considered to be edible and occurring worldwide. In France, since 1992 it has been incriminated in 12 intoxications in humans. The intoxication has been seen after repetitive ingestion of these mushrooms harvested in South West of France and is associated with 25% lethality. All subjects exhibited pure rhabdomyolysis without noticeable liver and kidney damage. The clinical pathology findings included large increases of circulating creatine kinase activity (CK) of up to 600 900 IU/L, Electromyography (EMG) and skeletal muscle histology confirmed the presence of rhabdomyolysis, without any neurological involvement. Using doses corresponding to mushroom quantities eaten by intoxicated patients, we have reproduced this intoxication in the mice by gavage with T. asperellum, the commercial mushroom Pleurotus ostreatus and p-phenylmercuric acetate was taken as controls. Only in mice fed T. asperellum, was circulating CK increased dose-dependently from 1401 to 1600 IU/L. This was confirmed by histological images of mild disorganisation of muscle fibers in surviving mice. Some mice died (about 30%) following the highest dose. ASAT, ALAT, LDH and creatininemia were determined and did not show significant increase. Since all other aseptic mushroom produces the similar tissue changes and death in animals we concluded that the consumption of large quantities of T. asperellum intoxication and since included that the consumption of large quantities of T. asperellum may indeed be harmful and or lethal in humans.

938
IN VITRO SCREENING OF CRUDE AQUEOUS PREPARATIONS OF WHOLE FOODS FOR POTENTIAL TOXICANTS.
G. D. Charles', V. A. Linscomb', B. Torres', J. L. Mattsson' and P. B. Gallapadi', TERC, The Dow Chemical Company, Midland, MI and 'Global Toxicology, Dow AgroSciences, Indianapolis, IN

The application of organic, conventional and molecular techniques may have the potential to alter the intrinsic levels of natural toxicants in crop plants. To maximize nutritional benefits and minimize the detoxification burden (metabolic cost) of ingesting crop foods, methods are needed to efficiently screen whole foods for unexpected changes in potential toxicity. We evaluated potential mammalian toxicity screens utilizing crude, aqueous preparations of whole foods purchased from a local market. Thirty-seven whole foods were evaluated (0.05-10% vol. extract/vol. culture medium) in order of the following tests: (1) general toxicity (cytotoxicity, all 37 foods) and (2) genetic toxicity (chromosomal aberration test, 9 foods) both performed in Chinese hamster ovary cells; (3) developmental toxicity (limb bud micromass assay, 9 foods) using 11-day old CD1 mouse embryos and (4) exposure to 50% of the maximal tolerated level (10% w/v). Five of the nine whole foods preparations (soybeans, broccoli, and lima beans) produced positive genotoxic responses compared to 17 beta-estradiol. Therefore, each of these tests showed some degree of response to these crude, whole foods extracts, and overall the 12 foods evaluated in two or more of the tests showed different patterns of response. These preliminary data indicate that screening for differences in potential toxicant profiles is possible with fast, relatively inexpensive in vitro tests. Funded by the Dow Chemical Company.

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FOUR-WEEK INHALATION STUDY WITH A MIXTURE OF DICHLORETHYLENE AND PERFLUOROBUTYLENE IN RATS.
L. A. Malley, J. P Hansen, N. E. Everds and D. B. Watter, DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, DE.

A study was conducted to determine the subchronic toxicity of a mixture of trans-1, 2-dichloroethylene (trans-DEE, 70%), cis-1, 2-dichloroethylene (cis-DEE, 30%), and perfluorobutylethane (PFBE, 25%). Groups of 10 male and 20 female rats were exposed by inhalation to 0, 400, 2000, or 8000 ppm of the mixture vapor for 6 hr/day, 5 days/week for 20 exposures. Subgroups of 10 males/group were observed during a 1-month recovery period. Functional observation battery (FOB) and motor activity (MA) evaluations were conducted prior to initiation of exposures, during week 4, and at the end of recovery. Clinical pathology evaluations were conducted during week 4 and recovery. At the end of exposure, and at the end of recovery, tissues were collected for microscopic evaluation. Decreased body weights and weight gains occurred in 8000 ppm males and females. Decreased food consumption and/or efficiency occurred in 8000 ppm males. During exposure to 8000 ppm, rats exhibited tremors and ataxia. Tremors were also observed during exposure period. Administration of the mixture vapor did not affect mice when administered to the eyes or by inhalation. Exposure to 8000 ppm had a diminished or no altering response to a sharp sound stimulus during the daily exposure. No evidence of compromised neurological function were detected when the rats were evaluated upon return to their cages following exposure. There were no test substance-related effects on FOB parameters or MA conducted the day following the last exposure period. In addition, there were no compound-related changes in clinical chemistry parameters. There were no compound-related gross or microscopic morphological changes. The mixture produced no functional effects during the exposure period which were not observed previously with either trans-DEE or PFBE alone. Although cis-DEE has been previously reported to cause neurological effects during exposure, the effects were observed at concentrations of 13, 500, 15, 700 ppm. Therefore, the mixture appears to have an additive or synergistic effect in producing transient neurological effects during exposure.

941
FOUR-WEEK INHALATION TOXICITY OF METHYLGLUTARONITRILE IN RATS.
G. L. Kennedy, D. P. Kelly, G. T. Makovec, S. R. Frame, L. A. Malley and N. E. Everds, Haskell Laboratory, DuPont Co., Newark, DE.

Methylglutaronitrile (MGN) is a high-boiling (263 °C) solvent/intermediate used in the fiber industry. Twenty male rats per group were exposed nose-only to approximately 5, 25, or 200 mg/m³ of MGN for 6 hours/day, 5 days/week over a 4 week period. Only a small amount of aerosol (<0.2 mg/m³ of MGN were present at the two lowest concentrations, the rats were exposed to 100 mg/m³ of aerosol was present at the highest concentration (MMAD 3.8 μm) along with 40 mg/m³ of vapor. Ten rats/group were sacrificed one day after the final exposure and the remaining rats after a four-week recovery period. Rats were given clinical, neurological, and histopathological examinations. No effects were observed in clinical observations during the exposure period, but body weight changes were observed in the 200 mg/m³ group. The 200 mg/m³ of MGN significantly reduced decrease in body weight. In the high level group returned to normal and there were no other clinical or pathological effects observed in any group. Based on these results, MGN was considered to have low toxicity in rats in repeated inhalation exposure.

942
FOUR-WEEK INHALATION TOXICITY OF N-VINYL CARBAZOLE IN RATS.
D. P. Kelly, G. T. Makovec and G. S. Ladies, Haskell Laboratory, DuPont Co., Newark, DE.

N-Vinyl carbazole (nVC) is a component of photo film. Male and female rats per group were exposed nose-only to concentrations of vapor for concentrations of 0.2, 9.5, of 44 mg/m³ of vapor for 6 hr/day, 5 days/week over a 4 week period. Rats were exposed to the same aerosol concentration of 0.6 and 0.4 mg/m³ of vapor, respectively. Ten rats/group were sacrificed one day after the final exposure and the remaining rats after a four-week recovery period. No effects were observed in clinical observations or body weight.
terminations during the exposure phase or recovery phase of the study. However, pathological effects were observed at all concentrations in nVC-exposed rats of both sexes. The effects were specific to the upper respiratory tract and consisted of degeneration/necrosis of olfactory epithelium, mucoid deploration of goblet cells, and squamous metaplasia and hyperplasia of respiratory epithelium in the anterior nasal passages and squamous metaplasia of the larynx. At the end of the four-week recovery period, the depletion of goblet cells in nasal cavities of female rats was no longer present, and was of decreased severity in the male rats, however, the olfactory epithelial degeneration/necrosis was still present in both sexes. Further work is underway to determine a no-effect level for nVC exposure in rats.

**943**

DIMETHYL GLUCARATE (DMG), DIMETHYL SUCCINATE (DMS), AND DIMETHYL ADIPATE (DMA): 90-DAY INHALATION TOXICITY STUDY IN RATS.


Groups of male and female rats were exposed to 0, 10, 50, or 500 mg/m³ DMG, 400 mg/m³ DMS, or 400 mg/m³ DMA for 6 hrs/day, 5 days/week over a 90-day period. The exposure period was followed by a 1-month recovery period. Rats were evaluated for clinical signs and growth throughout the study. Samples for hepatic, lung, and nasal cell proliferation (ICP) were collected from rats approximately 2 weeks and 90 days after study initiation. Clinical pathology, anatomic pathology, male reproductive endpoints, neurobehavioral assessments, neuropathological evaluation, estrous cycle, and hormonal analyses were conducted during the study. No compound-related effects were observed on mortality, clinical signs, clinical pathology, neurobehavioral endpoints, neurotoxicology, spermatocrit or spermiometry, or estrous cycle. Male rats exposed to 400 mg/m³ DMG had lower mean body weights and mean body weight gains during the study. Compound-related focal and minimal effects were observed in the nose of rats exposed to 400 mg/m³ DMG, DMS, or DMA for 90 days. CP in the nose of rats exposed to 400 mg/m³ DMG, DMS, or DMA was increased compared to controls during the study. Male rats exposed to DMS, serum testosterone concentrations were statistically significantly decreased at concentrations of 50 and 400 mg/m³. Serum luteinizing hormone concentrations were decreased in a dose-dependent manner and were statistically significantly decreased at 400 mg/m³. In female rats, DMG caused a statistically significant decrease in serum estradiol concentrations. There was an increase in epididymal sperm counts following exposure to DMG and the number of sperm per cauda and per gametes caudis epididymis was significantly increased at 50 and 400 mg/m³. In male rats exposed to DMS, epididymal sperm counts were significantly increased. In this study, the no-observed-effect level (NOEL) for repeated exposure to DMG was 10 mg/m³. NOELs for DMS and DMA were not established. Sponsored by the Dibasic Esters Group, Washington DC.

**944**

ROLE OF SENSORY NERVES IN THE AIRWAY AND CARDIAC RESPONSES TO SULFUR DIOXIDE (SO₂) IN A GUINEA PIG MODEL.

J. Stach1, Q. Q. Carter2, L. W. Wirters1, J. N. L. Carter1, W. Wirters1, W. W. Wirters1, and D. Costa1, College of Veterinary Medicine, NC State University, Raleigh, NC. School of Public Health, UNCG, Chapel Hill, NC, and Pulmonary Toxicology Branch, NIEHS, Research Triangle Park, NC.

Sulfur Dioxide (SO₂) is a common component of polluted urban air. Exposure to SO₂ has been shown to significantly alter airway compliance and resistance. Recent studies have shown that high concentrations of SO₂ during air pollution episodes have been associated with heart rate elevation. Using non-invasive methodology, we have previously shown that exposure to SO₂ produces a concentration-dependent increase in heart rate, the occurrence of which is significantly related to the severity of respiratory symptoms. The purpose of the current study is to determine the role of exposure to SO₂ produces simultaneous changes in airway and cardiac function and, 2) ascertain the role of sensory nerves in these responses. To this end, guinea pigs were surgically implanted with radioelectrometers capable of recording ECG, heart rate and core body temperature, and ³¹P data in 1-minute intervals. Animals were then exposed to either 0 or 100 ppm SO₂ in the head-space of a double-chamber plethysmograph. Airway and cardiac function were monitored continuously for a 10-minute baseline period and the 20-minute exposure period. Exposure to SO₂ produced a significant decrease in heart rate during the second half of the exposure period (p=0.05). To determine the role of sensory nerves in these responses, animals were pre-treated with the neurotoxic capsaicin (total dose 125 mg/kg, s.c.). DMG. A strain with capsaicin significantly diminished the SO₂-induced increase in SAWD and decreased in breathing frequency (p=0.05). In addition, capsaicin pretreatment attenuated the SO₂-induced decrease in heart during the second half of the exposure period (p=0.05). In summary, these results suggest that exposure to SO₂ produces simultaneous changes in both airway and cardiac function and that stimulus sensitivity of sensory nerves play a role in both of these responses. (Funded by the NCSSU Cooperative Education Training Program CT9206120101.) (This abstract does not reflect EPA policy.)

**945**

EFFECTS OF WELDING FUMES ON LUNG INJURY AND INFLAMMATION: THE POSSIBLE ROLE OF FREE RADICAL PRODUCTION.


The goals of this study were to examine lung damage and inflammation as well as free radical production caused by welding fumes of different composition. Fume was collected during flux-covered metal arc welding using stainless steel consumable electrode (MMA-SSE) and mild steel electrode (GMA-MS) or a stainless steel electrode (GMA-SS). Metal composition analysis by energy dispersive spectroscopy revealed that only MMA-SS was highly soluble, containing both soluble Cr and Mn, while GMA-MS and GMA-SS were relatively insoluble. Using electron spin resonance, hydroxyl radicals were observed in a suspension of MMA-SS fume containing hydrogen peroxide. Both GMA fumes showed evidence of free radical production. To examine the effects of the fumes on lung damage and inflammation, male Sprague-Dawley rats were intratracheally instilled with either a welding fume suspension at 2 mg/rat or the saline vehicle. On day 3, the right lung was assayed for lipid peroxidation (LPO) products while the left lungs were subjected to bronchoalveolar lavage (BAL). LPO products were elevated in the lungs following MMA-SS treatment only, implicating possible oxidative damage. All fumes caused increases in BAL cell numbers, including elevated macrophages and neutrophils. However, only MMA-SS led to an increased recovery of eosinophils, indicating a possible immune reaction. MMA-SS treatment caused an elevation of albumin in the first BAL fraction, indicating damage to the alveolar-capillary barrier. The concentration of thiobarbituric acid-reactive substance (TBARS) in the first BAL fraction was most elevated following MMA-SS treatment. These findings indicate that different welding fumes caused varied responses in the lungs of rats, and that these responses may correlate to the soluble metal composition of the fumes and the ability of the fume to produce free radicals.

**946**

TEMPORAL CHANGES IN BAL ELECTROLYTE LEVELS IN MICE AFTER EXPOSURE TO AN EDEMACINE GAS.


Detection of acute lung injury is important if therapeutic medical countermeasures are to be used to reduce toxicity in a timely manner. Indicators of injury may aid in the eventual treatment course and enhance the odds of a positive outcome following a toxic exposure. This study was designed to investigate the effects of a toxic exposure to the industrial irritant gas phosgene on the electrolyte levels in bronchoalveolar lavage (BAL) fluid. Phosgene (CG) is a well-known chemical intermediate capable of producing life-threatening pulmonary edema within hours after exposure. Four groups of 40 each CD-1 male mice were exposed whole-body to either A) no concentration x time (c x t) amount of 32 mg/m³ (8 ppm) CG for 20 min (640 mg/m³), BAL from A- or CG-exposed mice was taken at 1, 4, 8, 12, 24, 48, and 72 h postexposure. After euthanasia, the trachea was excised, and 800 μl of saline was instilled into the lungs. The lungs were washed 5x. Edematous lungs were placed in a cartridge and inserted into a clinical ISTAT analyzer. Na⁺, K⁺, Cl⁻, and ionized Ca²⁺ were analyzed within 2 minutes of insertion. The left lung was removed to determine wet weight (WW), an indicator of pulmonary edema. Temporal changes in BAL Na⁺ and Cl⁻ in CG mice were not statistically different from those in the A mice. Both Ca²⁺ and K⁺ levels were significantly higher than in the A mice over 72 h, p<0.05 and p<0.01, respectively. Significant changes in BAL Ca²⁺ and K⁺ occurred as early as 4 h postexposure in CG, p<0.05 versus A mice. CG caused a time-dependent significant increase in WW from 4-12 h, p<0.05, compared with A mice. BAL Ca²⁺ at 1 h in CG-exposed was 60% higher than in the time-point matched A mice. These data indicate that measuring BAL electrolyte levels may serve as an alternative to lung damage both Ca²⁺ and K⁺ follow-up parameters for early lung injury in tissue damage. In addition, knowledge of these levels may indicate how rational therapeutic intervention could be most beneficial.

**947**

OZONE EXPOSURE ENHANCES ANTIGEN-PRESENTING ACTIVITY ON BRONCHOALVEOLAR LAVAGE CELLS CONCENTRATION DEPENDENTLY IN RATS.

H. Watanabe1, T. Kikusui1, T. Kobayashi1. Department of Medical Sciences, University of Tsukuba, Tsukuba, Japan. 2 Department of Medicine, University of Tsukuba, Tsukuba, Japan, and Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Japan. Sponsor: S. Hirano.

Previous studies have shown that O₃ exposure aggravates allergy-like diseases such as asthma and rhinitis. Our previous studies have shown that expression of cell-surface molecules associated with antigen presentation on bronchoalveolar lavage cells

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(BAL cells) is increased by exposure to O$_3$ in rats. The present study designed to investigate concentration dependencies of the effect of O$_3$ on the expression of cell-surface molecules associated with antigen-presenting activity and on the accessory activity of BAL cells. Male Wistar rats were exposed to 0.3, 0.56, 1 ppm O$_3$ or filtered air for 3 days. Expression of cell-surface molecules (Ia, B7.1, B7.2, and CD11b/c) was measured by flow cytometry. Accessory activity of BAL cells was assessed by the allogeneic mixed lymphocyte reaction (MLR) and ovalbumin (OVA)-specific antigen-presenting activity. The numbers of Ia$^+$, CD11b$^+$, and Ia$^-$/CD11b$c$ BAL cells were increased by O$_3$ exposure concentration dependently. The accessory activity of BAL cells measured by MLR and OVA-specific antigen-presenting activity was also enhanced by O$_3$ exposure concentration dependently. These results suggest that the enhancement of antigen-presenting activity of BAL cells is caused by the increase in expression of cell-surface molecules associated with antigen presentation by O$_3$ exposure. This enhancement may trigger the subsequent immune response that could cause worse allergic asthma and rhinitis.

948 ULTRASTRUCTURAL CHANGES IN THE AIRWAYS OF RATS INHALING BUTTER FLAVORING VAPORS.

A. F. Hubbs, 1 R. R. Mercer, 1 L. Bartelli, 2 S. Friend, 1 D. Schwegler-Berry, 1 V. Castagnoli, 1 K. Kinnis, 1 G. Killman, 1 D. Froster, 1 W. T. Goldsmith 1 and W. G. Jones. 2 Health Effects Laboratory Division, NIOSH, CDC, Morgantown, WV, and 3Division of Respiratory Disease Studies, NIOSH, CDC, Morgantown, WV.

A cluster of eight cases of fixed airway obstruction occurred in former workers in a microwave popcorn plant. As part of a request to NIOSH to find the cause, extensive medical and environmental measurements were made at the plant. High levels of organic gases and decreased pulmonary function among workers were associated with butter flavoring exposure at the plant but other exposures existed. To assess the biological effects of the flavoring, we examined the effects by inhalation of rats to butter flavoring vapors. Qualitative GC-MS analysis yielded a variety of organic compounds including diacetyl and acetoin. Average diacetyl concentration in the exposure chamber was about 350 ppm. By transmission electron microscopy (TEM), the principal finding in the mainstem bronchus of exposed rats was airway epithelial necrosis. Unusual features of the epithelial reactions included rarefaction of basilar cytoplasm and relative preservation of cilia. Denuded basement membrane was frequently observed although a thin fragment of basilar cytoplasm of respiratory epithelium remained attached in some loci. Damage in the mainstem bronchus extended to the basal basement membrane into the lamina propria where edematous changes were characterized by disruption and separation of collagen fibers and fibrils. Necrotizing changes were less frequent in smaller airways. By scanning electron microscopy (SEM), ulceration of bronchiolar epithelium tended to be localized at bronchiolar bifurcations. No evidence of alveolar injury was evident in TEM or SEM sections. These findings document that artificial butter flavoring, while considered safe at concentrations present in food, produces vapors capable of inducing severe airway injury in laboratory animals when inhaled at high concentrations similar to peak exposures in the workplace.

949 SPECIES DIFFERENCE IN ACUTE RESPIRATORY RESPONSES TO VAPOR AND AEROSOL IRITANTS BETWEEN RATS AND MICE.


This study was to compare acute respiratory responses upon exposure to a vapor (cigarette smoke or an aerosol [valencium pentoxide, V$_2$O$_5$] irritant between young male F344 rats and SW mice. Animals (4/species/chemical/dose/timepoint) were exposed to either chemical and their tidal volume (TV), respiratory rate (RR), and minute volume (MV) were measured. For cuneate exposure, respiratory function was measured for 10-min preexposure and during 30-min nose-only inhalation exposure to 100 or 300 ppm cuneate. For V$_2$O$_5$ exposure, animals were exposed via intratracheal instillation (0.63, 2.63, or 6.3 mg V/kg in saline) and respiratory function was measured 1 and 3 days post dosing. For V$_2$O$_5$-instilled mice, bronchoalveolar lavage (BAL) was performed, and data were compared with those from V$_2$O$_5$-instilled rats under the same study design (Lee et al., 2000). Cuneate exposure: both concentrations significantly depressed TV and RR of mice, resulting in 39-41% reduction in MV compared to preexposure baseline. In rats, reduced respiratory function was observed only at 300 ppm (38% reduction in MV). For V$_2$O$_5$-instilled mice, respiratory became rapid (RR 170 and shallow (TV 4), which was most severe with the high-dose group on day 1 (MV reduced by 37% compared to saline-instilled controls). Signs of pulmonary inflammation (increases in LDH, NAG, protein, and neutrophils count in BAL fluid) were also observed in V$_2$O$_5$-instilled mice. For V$_2$O$_5$-instilled rats, rapid and shallow breathing was the most severe in the high-dose group on day 3 (MV reduced by 43%). Therefore, although acute respiratory responses between rats and mice varied depending on the exposure concentration and time, mice appeared to be more sensitive in terms of modifying respiratory functions or inducing inflammatory responses upon single exposure to these respiratory irritants.

950 INITIAL CONCENTRATIONS AND DISAPPEARANCE OF SELECTED MALODOROUS COMPOUNDS FROM FRESH AND AGED DAIRY PRODUCTS.

L. B. Willer, 1 D. C. Borger, 1 D. L. Elwell 1 and H. M. Keenert. 2 Animal Sci, Ohio State Univ, Wooster, OH and 3Food, Agricultural & Biol. Eng, Ohio State Univ, Wooster, OH.

Malodorous compounds from livestock manures are offensive and can cause health hazards to humans and cattle. Changes of manure storage and handling methods may prevent the formation and/or enhance the degradation of odors. This study was to quantify volatile fatty acids (VFAs), phenol, cresols, indoles and N$_2$H$_4$ in fresh and 12-day-aged manure. Loss of these compounds was monitored during pilot-scale composting in 205 L vessels either aerated continuously (AC) with high (2.3 kg/h) flow (0.8 kg/h) air flow controlled by thermostats or intermittently (AI) on 5 min high air flow 55 min off cycle. Manures were mixed with sawdust (1:1, w/w). Six replicates were conducted on each treatment combination, with 16 day trials. Emissions from the composting masses were extracts of solids. Each extract of phenolics and indolics were quantified by GCMS. Mean N$_2$H$_4$ emissions from fresh and aged manure AI were 50 and 60 g. Fresh and aged manure AC emitted 121 and 110 g N$_2$H$_4$. N$_2$H$_4$ peaked early and decreased to undetectable by day 17. Aged, compared to fresh manure, contained greater variety and amounts of VFAs, phenols and indoles with the most offensive odors. Fresh manure contained acetate, propionate, isobutyrate, isovalerate, phenol, p-cresol, and indole (5000, 500, 40, 70, 35, 200 and 5000 g/kg). Aged manure contained acetic acid, propionic acid, isobutyric acid, isovaleric acid, valeric acid, phenol, p-cresol, indole and skatole (9000, 2700, 200, 2500, 2800, 350, 500, 350, 10 and 30 g/kg). By day 8, trace quantities of acetate remained in fresh manure while acetic acid, propionate and butyrate (900, 120 and 130 g/kg) were in aged manure. Acetic acid, propionte and butyrate co-distilled with water being highest from aged manure. Bioprocesses associated with AC and AI influenced the loss of certain chemicals. Avoiding anaerobic aging of dairy manure was important in reducing concentrations of the chemicals studied.

951 WHOLE BODY EXPOSURE SYSTEM FOR ADMINISTERING NITRIC OXIDE (KINIX®) BY INHALATION TO NEONATE RATS.

T. J. Kenny 1, I. S. Gilkisson 1 and M. Lemair 1. Toxicology, Huntingdon Life Sciences, Huntingdon, United Kingdom and 2Air Liquide Sant International, Paris.

A whole body exposure system has been designed in order to expose neonate rats to controlled concentrations of nitric oxide for inhalation toxicity studies and to prevent levels of the oxidant product NO$_3$, rising above 1 ppm for an atmosphere containing 100 ppm NO. The exposure chambers comprise a polycarbonate nesting box with a perforated floor and a pyramidal lid incorporating a gas mixing chamber. Each exposure chamber accommodates a dam plus litter. A nitric oxide/nitrogen mixture of known concentration, oxygen and clean air are mixed and pass into the chamber via a duct in the chamber lid. The atmosphere is exhausted through the perforated floor. Gas flow rates are set to ensure a minimum residence time in the chamber to prevent NO$_2$ build up while maintaining temperature and relative humidity in the chamber according to the species of animals. The gas NO/NO$_2$/O$_2$ mixture, oxygen and airflow rates are controlled, monitored and recorded throughout exposure. An automated sampling and data capture system monitors the chamber concentrations of NO and NO$_2$ in real time. An inhalation toxicity study was conducted where neonatal rats were exposed for 6 hours a day with dams from Days 2–10 post-partum and litter alone from Days 11-29 post-partum, target levels of NO were 10, 30 and 100 ppm. Measured concentrations of NO were 10, 30 and 100 ppm. Concentrations of NO$_2$ were 0.0, 0.2 and 1.1 ppm. In conclusion, the study results demonstrate that the system is capable of excellent control of NO concentration and can maintain the required low levels of NO$_2$.

952 ALDEHYDE DEHYDROGENASE (ALDH2) POLYMORPHISM AND THE PULMONARY EFFECTS ASSOCIATED WITH EXPOSURE TO ETHANOL VAPORS IN THE RAT.

G. Del Zoppo 1, R. Tardi 2 and M. Charbonneault 1. Human Health Research Center, INRS-Institut Arriëns-Iriarte, Université de Montréal, Montréal, PQ, Canada and Environment and Occupational Health, Université de Montréal, Montréal, PQ, Canada.

Addition of ethanol to gasoline aiming at reducing the greenhouse effect is likely to increase atmospheric emissions of ethanol and acetaldehyde. By converting acetaldehyde to acetaldehyde 2 plays a major role in the metabolism and toxicity of EtOH. A significant polymorphism exists in both humans and rats. In the latter, a
point mutation gives rise to a slow metabolizing enzyme. This mutation is present on the ALDH2 allele but is absent on the ALDH2Q allele, with the ALDH2R allele expressing an incompletely dominant. Our hypothesis is that atmospheric ethanol may cause pulmonary toxicity and that individuals expressing the ALDH2R allele could be at greater risk. ALDH2 polymorphism was determined by PCR amplification of the polymorphic site followed by a digestion with Ddel enzyme. Characterization of the various genotypes in a group of Sprague-Dawley rats (Charles River Canada) (N=115) gave the following distribution: 2R/2R (17%), 2Q/2R (53%), 2Q/2Q (30%). Pulmonary ALDH2Q/2R enzyme showed greater in vitro metabolic activity, assessed by NADH formation, than ALDH2R/2R isozymes. Inhalation exposure experiments (500 ppm, 6h) showed that EOH is rapidly excreted from blood, i.e. around 94% of the dose is excreted in 60 min for female rats. Blood levels of acetaldehyde could not be detected in these rats suggesting its binding to proteins. Intratracheal instillation of 4C-EOH in female rats showed that after a 3 h period, ethanol-derived radiolabeled material binds to pulmonary proteins. Ongoing studies are aimed at determining if differences in amounts bound exist between ALDH2Q/2Q and ALDH2R/2R genotypes. Simulations between acetaldehyde and formaldehyde binding to proteins suggest that EOH vapors may lead to pulmonary toxicity. Overall, this work may improve the health risk assessment associated with the addition of ethanol to gasoline. (Supported by the Toxic Substances Research Initiative)

953
THE EFFECT OF COMBUSTION OF MOSQUITO COILS ON CHILDREN'S RESPIRATORY HEALTH.

We report a significant impact on the respiratory health of children due to combustion of mosquito coils. The groups of healthy Malaysian schoolchildren, ages 7-11, from 3 rural schools were chosen based on exposure to indoor air pollution. One group of 125 children came from homes in which there was cigarette smoking, home cooking twice or more daily, and the burning of insecticide-impregnated mosquito coils at night to protect children from mosquitoes. The control group of 156 children was negative for all of these characteristics. Pulmonary function studies showed that the FVC and FEV1 in girls and boys were each more than 10% lower in the exposed groups (p<0.001 for each). Respiratory symptoms were much higher in the exposed group: e.g. chronic cough was observed in 25/125 children in the exposed group vs. 7/156 in the exposed group (p<0.001). Further analysis showed that the pulmonary function and symptoms effects for the most part could be attributed to the mosquito coils. Studies of combustion products from mosquito coils from Malaysia and China revealed high levels of fine particulates and aldehydes. Coupling the observed emission rates with the range of expected room sizes and air exchange rates suggest that night time exposures to PM2.5 would range from 30 to 124, 340 ug/m3 (US and China) to 1 to 313.3 ug/m3; for formaldehyde 3.5 to 131.3 ug/m3; and for acetone 0.1 to 0.0.65 ug/m3. It is conceivable that at least 100 million children in Asia, Africa and Latin America are exposed indoors to burning mosquito coils.

954
RAT SUBCUTANEOUS INHALATION STUDY OF SMOKE FROM CIGARETTES CONTAINING FLUE-CURED TOBACCO CURED EITHER BY DIRECT-FIRED OR HEAT EXCHANGER CURING PROCESSES.

A subchronic nose-only inhalation study compared the effects of smoke from a cigarette containing 100% flue-cured tobacco cured by a direct-fired process to that of a cigarette consisting of 100% flue-cured tobacco cured by a heat exchanger process. The heat exchanger process applies indirect heat to the tobacco via a heat exchanger device. Sprague-Dawley male (M) and female (F) rats were exposed for 1 hour a day, 5 days per week, for 3 weeks to mainstream smoke at 0, 0.16, 0.20, or 0.80 mg/liter of air. Neither cigarette produced adverse changes in clinical signs, body or organ weights, serum chemistry, or histopathology. Carboxyhemoglobin and serum nicotine increases were both higher in cigarettes in an exposure-dependent manner. Smoked and histologic changes were nasal goblet cell hyper trophy, nasal mucus gland hyperplasia, and increased goblet cells in an exposure-dependent manner. Chronic/active inflammation and epithelial hyperplasia, chronic/active inflammation of the larynx, squamous metaplasia and acanthosis of the ventral larynx, tracheal epithelial hyperplasia and increased goblet cell contents, and increased goblet cells of the lung, and increased non-pigmented and brown/gold pigmented lung macrophages. When histologic changes resulting from exposure to smoke from the two types of cigarettes were compared, the only observed difference was increased epithelial hyperplasia of the anterior nasal cavity in males in the high exposure group for the heat exchanger cigarette. Since only one difference was noted among all of the observed smoke-related histologic changes, these findings indicate that the biological activity of smoke from cigarettes of flue-cured tobacco cured by a direct-fired or a heat exchanger curing process is not different under the conditions of the study.

955
CHRONIC EFFECTS OF INHALATION OF ROOM-AGED SIDESTREAM SMOKE AND DIESEL ENGINE EXHAUST IN THE RAT RESPIRATORY TRACT.

Environmental tobacco smoke (ETS) and Diesel engine exhaust (DEE) are widespread environmental combustion aerosols found at similar concentrations. Similar relative risks for lung cancer have been reported for both. The purpose of this investigation was to determine the carcinogenic potency of room-aged sidestream smoke (RASS), as a surrogate for ETS, and DEE at toxicologically relevant concentrations that bear a realistic relationship to human exposure. Male and female Sprague-Dawley rats were exposed to fresh air for 6 months followed by fresh air and DEE for 6 months followed by DEE at 3 and 10 mg/m³ for 6 days/7 week for 24 months followed by a 6-month postinhalation period. To increase the sensitivity for the detection of lung tumors, 0.5 mm step serial sections were utilized. The only findings in the nose, larynx, and trachea were hyperplasia and metaplasia. In the lung a few RASS-related non-neoplastic findings (goblet cell hyperplasia, pigmented alveolar macrophages) were observed. There was a dose-dependent increase in the frequency of DEE-associated non-neoplastic changes, which consisted of alveolar epithelial hyperplasia, bronchoalveolar metaplasia, squamous metaplasia, alveolar, interstitial, and pleural particle-filled macrophages particulate matter, chronic active inflammation, and sebaceous fibrosis. The incidence of lung tumors (102 rats/group) in the RASS-exposed groups was 5% each (adenomas and one adenocarcinoma) and not statistically significantly different from sham (2%). In DEE-exposed groups lung tumors first appeared between 12 and 24 months. The incidence of tumors increased with dose (up to 50%) and comprised benign cystic tumors, adenomas, adenocarcinomas, and squamous cell carcinomas. This spectrum of neoplastic and non-neoplastic findings in rats exposed to DEE is consistent with the literature. No alveolar hyperplasia was seen at any time point in the RASS groups, which suggest that the tumors in the RASS groups were spontaneous in nature. INIBIO is a Philip Morris research laboratory.

956
NEUROKININ RECEPTOR-1 GENE EXPRESSION IN MICE SUBJECTED TO SIDESTREAM CIGARETTE SMOKE.

There are unmyelinated afferent nerves (C-fibers) that serve as an important sensor in detecting the onset of pathophysiological changes in the lungs. To investigate whether the gene activation of neurokinin receptor-1 (NK-1) following sidestream cigarette smoke (SSCS) exposure, the elderly C57BL/6J mice were randomly assigned to 5 groups (10/group) in an effort to explore the dose-effect relationship. The mice were exposed to smoke for 8 hours per day, 5 days per week, for 8 weeks. The expression of NK-1 mRNA in the lung, heart, liver, kidney, and spleen tissues were detected by RT-PCR techniques and normalized against GAPDH expression. The mice subjected to lower techniques and normalized against GAPDH expression. The mice subjected to lower SSCS exposure had a downregulation of NK-1 mRNA expression in heart, but an upregulation in liver and kidney when compared to control rats. However, less or more induction for the NK-1 gene was observed following the higher dosages of SSCS exposure when compared to the lower dose of SSCS exposure in these tissues. This finding suggests that there are the paracrine and/or autocrine signaling mechanisms through receptor-ligand interactions. No alteration of NK-1 gene expression was observed in the lung and spleen tissue in this study. In conclusion, alteration of NK-1 gene expression may contribute to passive cigarette smoke-induced lung responses, which may be of primary importance in shifting neurogenic inflammatory responses from their physiological protective/defense functions to detrimental pathophysiological roles (Supported by ADBCRC No. 9925).

957
REVERSALS OF B-CHEMOKINE RECEPTOR GENE EXPRESSION IN C57BL/6 MICE EXPOSED BY SIDESTREAM CIGARETTE SMOKE.

Chemokines have recently been shown to play an important role in the activation and directional migration of inflammatory cells to sites of tissue injury. Their action requires the expression of their complementary chemokine receptors by their target.
cells. However, the possible action of B-chemokine receptors (CCR) genes in the pathogenesis of environmental tobacco smoke has not been established. To examine the expression of CCR genes in the lung, heart, kidney, and spleen tissues were isolated from C57BL/6 mice, which are daily exposed to sidestream cigarette smoke (SSCS). From the (room air), 2, 4, 8, and 16 standard expression of CCR-1 to CCR-5 genes were detected by RT multiplex PCR techniques and normalized against GAPDH expression. In SSCS-exposed CCR-1 to CCR-3 mRNA levels of mice exposed to lower SSCS concentrations basically presented a decreased trend in the lung, heart, and kidney tissues, but an increased trend in liver and spleen tissues. However, significant reverses for these CCR gene expressions in heart and kidney tissues were observed following the higher dosages of SSCS exposure, suggesting there are the paracrine and/or autocrine signaling mechanisms through receptor-ligand interactions. The changes in CCR gene expressions in the tissue suggests the B-chemokine receptor and its ligand, such as eotaxin-1, eotaxin-2, eotaxin-3, RANTES, MCP-2, 3, 4, and MIP-3, may play an important role in the recruitment of inflammatory cells following SSCS exposure. (Supported by ADRC No. 9925)

**957A**

*OZONE TOXICITY IS ASSOCIATED WITH UPREGULATION OF PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) AND PROTEIN KINASE B (PKB) IN LUNG MACROPHAGES. ROLE OF CAVOLIN-1.*


Exposure of mice to toxic levels of ozone (O3) causes type I epithelial cell damage and type II cell proliferation. This has been associated with increased expression of inducible nitric oxide synthase (NOS1) and production of reactive nitrogen intermediates by alveolar macrophages (AM), which have been implicated in tissue damage. In the present studies, we have analyzed mechanisms regulating NOS1 activity in AM following O3 exposure. The promoter region of the NOS1 gene is controlled by several transcription factors including NF-KB, O2- activation (0.8 ppm, 3 h) caused a time-dependent induction of NF-KB nuclear binding activity in AM. The findings that AM isolated from Control mice lacking the p50 subunit of NF-KB did not generate nitric oxide, and that the mice were protected from toxicity demonstrate that signaling through NF-KB is important in macrophage lung injury. Activation of NF-KB requires phosphorylation of IKB kinase, kinase, as well as NF-KB subunit proteins. IKB is phosphorylated in a time dependent induction of phosphatidylinositol-3′-kinase (PI3K) and its downstream target Akt/protein kinase B (PKB) in AM. Moreover, treatment of the cells with the PKB inhibitors, Wortmannin and LY294002, blocked nitric oxide production. This was correlated with reduced NF-KB nuclear binding activity in the cells. PI3K activity is controlled by cavelolin, a family of highly conserved integral membrane proteins. We found that cavelolin-1 (Cav-1) was constitutively expressed in AM. O2- exposure caused a marked reduction in Cav-1 in AM, which was evident immediately following exposure and persisted for 6 h. Changes in expression of Cav-1 were inversely correlated with expression of PI3K and PKB in AM. Taken together, these data suggest that Cav-1 may play a role in PI3K and PKB signaling and NF-KB activation leading to nitric oxide production following O2- activation. Supported by NIH grants ES04738 and ES06897.

**958**

*A METABOYKIN APPROACH TO STUDY THE ONSET OF NEUROTOXICITY USING 3-NITROPROPIONIC ACID, A MODEL FOR HUNTINGTON’S DISEASE.*


The model toxin 3-nitropropionic acid (3-NP) replicates in animals, most of the pathological features, cognitive and motor dysfunctions that are observed in humans with Huntington’s Disease [1]. We have used a metabolic approach to study the onset of toxicity in male Sprague-Dawley rats (<10 per group) following daily dosing with 3-NP (7.5 mg/kg/day) over a 14 day period. Urine samples were collected throughout the study for NMR spectroscopic analysis and histological examinations were carried out on all animals at study termination. Rats treated with 3-NP exhibited weight loss, reduced food and water consumption and a degree of functional impairment. Although the animals exhibited symptoms ranging from ataxia to right hind-limb paralysis in all dose groups, histology failed to show the expected neuronal loss in brain tissue. Clinical findings were accompanied by mild to moderate decreases in urinary pH from 8.5 to 7.4. However, NMR analysis demonstrated a marked increase in the excretion of urinary succinate immediately post-dose in these animals. The results were accompanied by mild to moderate decreases in urinary pH from 8.5 to 7.4. However, NMR analysis demonstrated a marked increase in the excretion of urinary succinate immediately post-dose in these animals. Multivariate statistical analysis using principal components analysis (PCA) confirmed an increase in succinate as the predominant biochemical change and demonstrated a Dose-Response relationship in the separation of 3-NP treated animals from controls. The increase in urinary succinate excretion following administration of 3-NP has not been reported previously, but is consistent with the observation that 3-NP is an irreversible inhibitor of succinate dehydrogenase [2]. This result suggests a gross impairment of energy metabolism in the TCA cycle, which could be the cause of neuronal cell death. The observation of increased succinate in the absence of the expected brain pathology demonstrates the utility of NMR in these disease and the metabolomic approach as an adjunct to standard toxicological techniques, particularly during the onset of toxicity. 1. Acta Medica 43, 9-13 (2000) 2. Journal of Biological Chemistry 254, 5161-5167 (1979)

**959**

*EFFET OF DEXAMETHASONE ON THE METABOLIC PROFILE ASSOCIATED WITH PHOSPHODIESTERASE (PDE) INHIBITOR-INDUCED MESENTERIC VASCULAR LESIONS IN RATS.*


Metabolomics is a novel technology proposed as a predictive tool for screening novel compounds and for identification of biomarkers of toxicity in biological fluids. Utilizing this approach, we previously identified urinary spectral patterns in rat characteristic of vascular lesions induced by the suprapharmacological doses of the type IV PDE inhibitor CI-1018. Whether the observed changes in metabolomic profile were related to the mechanism of toxicity or were instead a measure of the subsequent inflammatory response was uncertain. In the present study, dexamethasone (DEX) was used to examine the relationship between the inflammatory process and the metabolomics profile induced by CI-1018. Female Sprague-Dawley rats were given 5 daily doses of DEX (1 mg/kg IP), 4 daily doses of CI-1018 (750 mg/kg PO), or given a placebo of DEX followed by 4 daily doses of DEX plus CI-1018. Twenty four hour urine samples were collected from each rat daily for the duration of the study for metabolomic analysis. All rats doused with CI-1018 alone had marked mesenteric and mild hepatic vascular lesions characterized by medial necrosis, hemorrhage and/or necrosis accompanied by perivascular infiltrated inflammatory cell infiltrates. Minimal arteriopathy without concurrent inflammatory infiltration was noted in 5 of 6 rats given both dexamethasone and CI-1018. Principal component analyses performed on urine samples revealed an obvious pattern of separation between samples from control rats versus those from CI-1018-treated rats with marked lesions and rats given DEX plus CI-1018 with minimal lesions and no inflammatory cell infiltration. Pattern separation was not evident between the two CI-1018 treatment groups. The separation from controls, observed in rats with minimal lesions without inflammatory infiltrates, suggests that the observed shift in spectral pattern is independent of the inflammatory process associated with vascular injury induced by PDE inhibition.

**960**

*HEPATIC OXIDATIVE STRESS RESPONSE TO LAZABEMIDE.*


Tembium® (Lazabemide: Ro 19-6327) is a potent, highly selective and reversible inhibitor of MAC-B with clinical advantages over current therapies for the treatment of Alzheimer’s disease. During the pre-clinical safety program an increase in rat liver enzymes with some similarity to clinical findings was observed. In a limited number of patients, transient increases in ALT and GGTT, and less frequently AST, have been recorded between weeks 6 to 12 of lazabemide treatment (100 mg bid). In one patient, however, plasma enzymes continued to increase after withdrawal of treatment but eventually declined to reference levels. Based upon this data and the pre-clinical findings the transient and reversible increase in liver-related enzymes appears to affect patients and experimental rats alike. Further investigative work was initiated to address the mechanism of this hepatic response. NMR analysis (metabolomics) of urine samples from rats treated with lazabemide (1500 mg/kg) indicated a decreased excretion of succinate, creatine and oxaloacetate consistent with disruption of mitochondrial respiration (see presentation by U. Niederhuthe et al.). As lazabemide is also a substrate for mitochondrial monoamine oxidase it was hypothesized that lazabemide induced hepatotoxicity is related to oxidative stress conditions likely due to a production of reactive products capable of mitochondrial function impairment. Treatment of rat hepatocytes in primary culture with 300 μM lazabemide did not lead to measurable cytoxic effects. However, pretreatment of cells with buthionine-sulfoximine and diethylmaleate depleted cellular glutathione and inhibiting its synthesis markedly increased cytoxicity as assessed by the release of lactate dehydrogenase into the incubation medium. Thus, reduced glutathione appears to play an essential protective role against lazabemide-mediated cytotoxicity. Additional experiments are at detecting reactive species originating from lazabemide and its metabolism will be presented to clarify the role of oxidative stress response on mitochondrial function.

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961 METABONOMICS STUDY INTO THE BIOCHEMICAL EFFECTS OF RO 19-6327 USING 1H NMR SPECTROSCOPY AND PATTERN RECOGNITION ANALYSIS OF URINE SAMPLES.


Tempusro (Larabemide; Ro 19-6327) is a potent, highly-selective and reversible inhibitor of MAO-B with possible clinical advantages over current therapies for the treatment of Alzheimer's Disease. Results of toxicity studies showed that Ro 19-6327 induces in rats after respective oral doses of 700 mg/kg a mild transient increase in liver enzymes (mainly ALT) and an increase in liver weight. A similar trend for an increase in liver enzymes was observed in patients. To evaluate the technique and to gain further insight into the effects of Ro 19-6327, 1H NMR spectroscopy and metabolomics investigations have been performed using urine samples of Wistar rats. Two dose levels (single oral doses of 600 mg/kg and 2000 mg/kg) were given to assess the sensitivity of the metabolomics approach, and to evaluate if there are quantitative as well as qualitative changes in the toxicity profile. Ro19-6327 caused subtle biochemical perturbations in the urinary 1H NMR spectra. The major changes in the urinary profiles were, beside the appearance of drug related resonances, severe reduction of citrate, 2-oxoglutarate and succinate. Depletion of these TCA cycle intermediates may be indicative of mitochondrial dysfunction. The subtle elevation of creatinine and uric acid excretion may be associated with effects of Ro19-6327 on the liver. Little difference in the biochemical response of rats treated with 600 or 2000 mg/kg of Ro 19-6327 was observed. However, rats treated at the higher dose showed a more prolonged response. The effects were quickly reversible, with urinary profiles returning to normal within 4 days post-dose. Conclusion: Metabolomics may add valuable information to assessment drug-induced effects. The results also confirm that Ro19-6327 exhibits low toxicity in the rat.

962 IMPROVED CLASSIFICATION OF HEPATIC TOXICITY BASED ON PATTERN RECOGNITION INVESTIGATION OF DIFFUSION-EDITED 1H NMR SPECTRA OF BLOOD SERUM.


Chemometric investigation of 1H NMR biofluid spectra taken from animals dosed with model toxins generates characteristic patterns of metabolic responses and enables the identification of biomarkers of toxic response and regeneration. Most of the studies to date have been conducted on urine, with limited investigations using blood plasma. In the current study we demonstrate new experimental methods for investigating toxin-induced changes in plasma profiles using 1H NMR (diffusion edited and 1H NMR) spectra. The use of conventional 1-dimensional 1H NMR spectra generates complex spectra with each biofluid requiring tailored NMR experiments and processing to optimize biological interpretation. Biofluids such as blood plasma or serum contain a wide range of macromolecules that generate a broad envelope of NMR signals, on which are superimposed sharper signals from lower mw metabolites and thus, key biomarker metabolite resonances can be obscured. ANIT was administered to male SD rats (n=20 mg/kg p.o.) and plasma samples obtained prior to and at 48 and 72 h post dosing. In order to simplify the subtle ANIT-induced changes in the lipidprotein profile of serum, diffusion edited spectroscopy coupled with principal components analysis was used to focus on toxin-induced lipoprotein changes by minimizing the contribution of low mw molecules to the spectral profile. Using diffusion edited spectra, marked changes to the plasma lipid profiles were detected and good separation of control and treated animals was effected. This study has demonstrated the potential of the application of chemometric analysis to diffusion edited 1H NMR spectra for probing lipoprotein metabolism disorders associated with toxic lesions.

963 NMR-BASED METABONOMICS STUDIES ON SALT TOXICITY IN ZEA MAYS: DECONVOLUTION OF PHYSIOLOGICAL AND TOXICOLOGICAL FACTORS.


High salinity, whether natural or induced by agricultural methods, is a widespread environmental stressor that can affect development and growth of plants. We have shown that application of NMR-based metabonomics technology can be used to characterise saline-induced stress effects in Zea Mays and to identify pertinent biomarkers. Interpretation of NMR-based metabolomic toxicity studies may be confounded by non-related physiological metabolic variation. Statistical experimental design was used to examine the relationship between metabolic differences arising from growth effects and salt toxicity. Manze plants were subjected to a continuous dosing of either 0, 50 and 150 mM saline solution and harvested after 2, 4 and 6 days (n=5 per class). 1H NMR spectroscopic analysis of the root and shoot extracts indicated differences in the metabolite spectral profiles relating to saline-induced stress. Clustering of control and treated classes based on inherent biochemical differences in the saline-treated plants was achieved via application of Principal Components Analysis to the spectral data. Analysis of the control and treated groups revealed clustering according to the time of harvesting, indicating differences in the metabolite profile resulting from growth effects. Partial Least Squares Analysis was employed to deconvolve the biochemical effects of growth and salt stress. Examination of the regression coefficients revealed important metabolic markers for saline toxicity including perturbed levels of malic acid, glucose, alanine, succrose and glycinebetaine. This NMR-based metabolomic technology provided an efficient method for identifying stress effects in plants. Furthermore, statistically significant biomarkers for salt toxicity, growth differences and the interaction of these physiological factors were identified.

964 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC AND PATTERN RECOGNITION STUDIES OF 3-NITROPROPIONIC ACID-INDUCED NEURONAL DAMAGE.

T. M. Tsang, J. Griffin, J. N. Haselden, M. L. Anthony, J. K. Nicholson and E. Holmes, 1 Biological Chemistry, ICMS London University, South Kensington, London, United Kingdom and Safety Assessment, GSK Pharmaceuticals, Ware, Herts, United Kingdom. Sponsor: D. Robertson.

3-Nitropropionic acid (3NP) is a neurotoxin that produces lesions within the basal ganglia (particularly targeting the striatum) in rats and non-human primates with symptoms mimicking Huntington's Disease. 3NP is also an irreversible inhibitor of succinate dehydrogenase. Nuclear magnetic resonance (NMR) spectroscopy combined with pattern recognition (PR) analysis of biofluids and tissues offers a powerful approach to the investigation of the molecular consequences of toxin exposure. In this study magnetic resonance spectroscopy and PR methods were applied to characterise the metabolic alterations in various neuronal regions caused by 14 days repeated exposure to 3NP (0, 5.0 and 7.5 mg/kg i.p.) in the Sprague-Dawley rat. Selected brain regions were dissected and immediately snap frozen before analysis by 1H MAS-NMR. Principal components analysis (PCA) was used to show the biochemical variations induced by 3NP toxicity as a time-related trajectory plot. From the PCA eigenvector loadings, the discriminating regions of the NMR spectra and thus the contributory endogenous metabolites were identified. Reductions in lactate, acetate, NAA, GABA and creatine levels were observed together with increases in succinate following systemic administration of this toxin. These studies show that MAS-NMR PR offers a powerful contribution for the investigation of basic mechanisms of neurotoxicity.

965 COMPARISON OF THE TEMPORAL TOXICITY OF HYDRAZINE IN RAT AND MOUSE USING NMR-BASED METABONOMICS.


As part of our ongoing Co-ordinating Centre for Metabonomic Toxicology (COMET) designed to systematically investigate metabolic effects of drugs, 1H NMR spectroscopic and pattern recognition (PR)-based methods were used to investigate the biochemical responses to toxins in rat and mouse models. Principal components analysis (PCA) was used to evaluate the metabolic effect of hydrazine at 30 and 90 mg/kg in the rat and at 100 and 250 mg/kg in the mouse. Urine samples were collected from dosed and control animals at 10 time-points over an 8 day period and analysed by 600 MHz 1H NMR spectroscopy. Urinary biomarkers of hydrazine toxicity of the two species were determined from the PC loadings and included a number of endogenous metabolites which were common to both, including 2-aminoadipate, succine, creatine and the TCA cycle intermediates. In the mouse, toxicological trajectories were mapped in PC space using the spectral data at each time point providing a means of monitoring the progression of and recovery from the toxic lesion. Due to the disparate magnitude of response shown in the two species, it was difficult to combine the two data sets in a single chemometric model. Thus, a hierarchical method was developed using multiple partial least squares (PLS) regression models which took account only of the section of response in metabolomic space rather than magnitude. This enabled the time course of the responses to be compared in a meaningful way showing that, although there were many similarities in

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the effect of hydratone dosing on endogenous metabolites, there were also subtle differences between the two species. Incorporation of these chemometric methods into automated NMR-based metabolomics will enable toxicological screening of biofluids from different species, thus aiding determination of the model species that best mimic the human.

966 A METABONOMIC INVESTIGATION OF THE BIOCHEMICAL EFFECTS OF ROSIGLITAZONE AND WY14643.
1 Biological Chemistry, ICMS London University, South Kensington, London, United Kingdom and 2 Drug metabolism, Novo Nordisk, Malun, Denmark. Sponsor: D. Robertson.

The effects of diabetic treatment drugs Rosiglitazone and WY14643 were investigated by NMR-based metabolomic analysis using groups of healthy male Sprague-Dawley rats (n=10). 1H NMR spectra of plasma samples were obtained and data filtering and pattern recognition analysis applied to the spectra, in order to establish baseline reference effects of these drugs with particular emphasis on potential toxicological responses in non-disease state animals. Samples obtained after a low dose of Rosiglitazone administration were differentiated from the control group by increases in several metabolites including lipoproteins, triglycerides and glucose, whereas the intermediate group reversed this change and was most similar to the control group. The high dose group presented changes in the opposite metabolic direction to that of the low dose group, with lowered lipids and glucose levels. The WY14643 response also exhibited differential dose-related effects, initially at low dose showing increased lipids and decreased glucose signals. The intermediate dose group showed an effective reversal of this pattern, and the high dose group was the closest to the control group, though marginally higher in glucose, choline and VLDL levels. This metabolomic evaluation of drug response is aimed at allowing a better understanding and characterisation of the metabolic responses of disease animals to these drug treatments, and in particular to identify potential biomarkers of toxic responses in the metabolite of healthy individuals.

967 BUILDING NMR SPECTROSCOPIC METABONOMIC DATABASES OF XENOBIOTIC TOXICITY.
Biological Chemistry, Biomedical Sciences, Imperial College Faculty of Medicine, London, United Kingdom.

The Co-Ordination for Metabolic Toxicology (COMET) project will construct a database of drug toxicity using ca. 100,000 1H NMR spectra of biofluids from animals treated with model compounds. Mathematical models characterising the effects on endogenous metabolites will enable rapid toxicological screening of potential drug candidates and discovery of novel mechanisms and biomarkers for specific types of toxicity. To reveal any systematic differences between the 7 participating animal study centres, hydratone was administered orally to rats at each site, in two dose groups (30 and 90 mg/kg). Urine samples were collected from dosed and control animals at 10 time-points over an 8 day period. At Imperial College, the urine was analysed by 600 MHz 1H NMR spectroscopy and the spectra used to build a statistical pattern recognition (PR) models based on principal component analysis (PCA) and partial least squares (PLS), describing both dose, time and between-Lab-related spectral signatures. The resulting 7 independent datasets showed a high degree of similarity, yet the metabolomic techniques could detect genuine low level biological differences between the laboratories. In addition, 1H NMR spectra of plasma samples collected at 48 and 168 h post dose were analysed using PR. To further refine the information retrieval, PLS was used to model the relation between the NMR spectra and clinical chemistry of the plasma. This revealed co-variation between the NMR and clinical chemistry data, facilitating more detailed interpretation of the metabolomic variation. Finally, the reproducibility of metabolomic methodology was assessed by comparing spectra acquired at two different NMR facilities from aliquots of the same urine samples. The two data sets showed close similarity, confirming the robustness of the technique. This work illustrates that metabolomic studies can obtain novel, reproducible information on the toxicity of model, and inevitably new compounds, across multiple laboratories with large numbers of samples.

968 MINIATURIZED NMR PROBEHEAD WITH INCREASED SENSITIVITY FOR PROFILING BIOFLUIDS AND METABOLITE IDENTIFICATION.
G. Scholterbeck1, A. Rosse1, H. Senn1, D. Marek1, T. Kuehn1 and O. Scherz. 1BHS-I, F. Hoffmann-La Roche, Basel, Switzerland and 2Bruker BioSpin AG, Fällanden, Switzerland. Sponsor: D. Robertson.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful analytical tools to determine the structure of small molecules. It suffers, however, compared to other analytical techniques, from an inherent low sensitivity. Structural characterisation of mass- and volume limited samples are difficult with conventional NMR detectors, e.g.: biofluids (CSF, urine, plasma and synovial fluids), drug metabolites, fractionated peaks from micro-separation techniques and isolates of natural products. A miniaturised 1mm NMR probe was built with a 100-fold smaller active sample volume than conventional NMR probeheads. The new probehead has an active volume of 2.5 μL. With this probehead, a high resolution NMR spectra was performed with hitherto unobtainable mass sensitivity. The improvement in the signal to noise ratio by a factor of 4 compared to a conventional system lead to a reduction in measuring time of more than a factor of 16. Furthermore the favouable ratio of analyte to solvent lead to spectra with very low solvent back- ground. NMR results are shown which illustrate the performance of the new probehead for metabolomics investigations and metabolite identification. These include 1D 1H NMR spectra of rat urine, plasma and CSF, and 2D NMR experiments of mass limited samples.

969 APPLICATION OF METABONIMICS TO PHARMACEUTICAL DRUG DEVELOPMENT AND EVALUATION OF PHENYLACETYLGLYCYLNE AS A POTENTIAL MARKER OF PHOSPHOLIPID ACCUMULATION.

Phospholipidosis (PLDs) can be caused by a range of agents in many animal species, including man [1]. PLDs is characterised by the accumulation of phospholipid-rich lamellated and crystallloid inclusions, identifiable only by transmission electron microscopy within a variety of cells [1, 2]. PLDs is a frequent finding in toxicology studies and, in order to minimise impact on drug development, there is a need to develop less labour-intensive and time-consuming methods for its detection. We have used nuclear magnetic resonance (NMR) spectroscopy to help identify biochemical markers in urine collected from Sprague-Dawley rats following 7 days' repeated dosing with six structurally-related drug candidates (the cationic amphiphiles GSK-A to F) at 3 dose levels. All six compounds in the screen demonstrated morphological changes indicative of phospholipid accumulation in the mesangial area at the high dose level. Although a number of general housekeeping biochemicals were shown to change in urine by NMR, a feature common to all six drug candidates was a rise in the urinary levels of phenylacetylglycine (PAG). This observation supports previous reports that PAG may constitute a potential marker of PLDs [3, 4]. Significantly, PAG was also present in increased amounts in urine collected at 3 days for 6 months with a structurally-unrelated drug candidate, GSK-G, that caused PLDs. In this case, PAG returned to control levels in urine collected from these animals after a 3 month off-dose period, mirroring a reversal of PLDs as detected by electron microscopy. This study highlights the utility of metabolomics technology in the monitoring of PLDs as part of novel chemical entity evaluation. Studies are ongoing to elucidate the origin of PAG and its biochemical significance. 1. Toxicologic Pathology 25, 53-60 (1997) 2. CRC Critical Reviews in Toxicology November, 185-217 (1973) 3. Biomarkers 5, 410-423 (2000) 4. Magnetic Resonance in Chemistry 39, 559-565 (2001)

970 APPLICATION OF METABONIMICS IN DRUG DESIGN: CAN METABONIMICS AND PHARMACOKINETIC (PK) STUDIES BE CONDUCTED SIMULTANEOUSLY IN THE SAME SET OF RATS?

High-resolution 1H-NMR spectroscopic analysis of biofluids is a powerful technique for investigating the response of organisms to xenobiotics. The 1H-NMR spectral profiles of biofluids provide a unique temporal fingerprint of the metabolic state of an organism and information on the nature of xenobiotics to which an organism has been exposed. Metabonomics methods combine high resolution NMR of biofluids with pattern recognition techniques to determine the onset, duration, and localization of a toxic lesion in target organs. The feasibility of simultaneous metabolomics and PK studies in the same rats was assessed. Male Sprague-Dawley rats were separated into two experimenal groups, without surgery (normal) or canulated in the carotid artery (canulated). Within each group, rats (n=6) received vehicle (control), methotrexate, or cactaminogen. Blank urine was collected on day 0. On day 1, treatments were delivered and the canulated group was subjected to PK bleeding procedures. Urine was collected twice a day for 7 days. Urinary NMR spectra were acquired and data were analyzed with principal component analysis (PCA) and soft independent modeling of class analog (SIMCA). Good clustering and good separation of clusters were observed in PCA map for all groups. When treated with xenobiotics, canulated rats do not cluster the same as normal rats, indicating surgery had an impact on the response to xenobiotics treatment.
971 COMPARISON OF NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF URINE AND CONVENTIONAL PATHOLOGICAL MEASUREMENTS IN PRECLINICAL SAFETY EVALUATION OF ALLYL ALCOHOL


Detection of organ specific toxicity using nuclear magnetic resonance (NMR) spectroscopy of urine may be a novel method for evaluating toxicity of xenobiotics. This technique may allow for detection of toxicity below the doses that induces changes in standard pathology endpoints (e.g., serum chemistry and histopathology). The present study assessed this proposition using the model hepatotoxicant, allyl alcohol. Four Sprague-Dawley rats of each sex were orally gavaged with allyl alcohol at 0, 60, 120, or 240 mg/kg on Day 1 of the study. Survival permitting, two animals of each sex were necropsied on Days 3 and 5. Twenty-four hour urine samples were collected in metabolic cages prior to dosing and daily through Day 5. NMR spectra were acquired and the data subjected to principal component analysis (PCA). At necropsy, blood was taken for serum chemistry analysis, and selected organs were processed for microscopic evaluation. Allyl alcohol induced significant, dose-dependent increases in total bilirubin, blood urea nitrogen, release of hepatocyte cell enzymes (ALT, AST, LDH), and globulin as well as reducing serum albumin. The three most common histologic lesions were hepatic necrosis, forestomach ulceration, and granulocytic hyperplasia. NMR analysis of urine reliably separated samples from treated animals from those of control and pre-dose samples. The PCA of hematologic and clinical chemistry showed similar pattern without time-dependent information. Allyl alcohol (60 mg/kg) increased urinary tubae size on Day 1, with partial recovery by Day 5. The extent of NMR changes was correlated with the severity of hepatocellular necrosis. The NMR PCA revealed that inter-animal differences exceeded intra-animal differences. These data suggest two main roles for NMR spectroscopy in preclinical safety assessment: non-invasive metabolic analysis of novel xenobiotic toxicity, and selection of metabolically homogenous animal population for in vivo studies.

972 EVALUATION OF METABONOMIC TECHNOLOGY FOR LIVER AND KIDNEY TOXICANTS IN MICE


Metabonomics technology has been well characterized for the rat, however limited information is available for mice. The purpose of this study was to evaluate the utility of the mouse as a test system for early toxicity screening using metabonomics. Caplinin (CP), 2-hydroxymethylamine (BEA), carbon tetraclorid (CCL), and p-aminophenol (PAP) were used as representative liver and kidney toxicants. Male B6C3F1 mice (4/group) were individually housed in metabolism cages and urine collected pre-test and every 24 hr period through Day 8. A low and high dose for each toxicant was selected to assess a Dose-Response effect. CP (10 and 25 mg/kg), BEA (100 and 300 mg/kg), CCL (500 and 2000 mg/kg) and PAP (100 and 300 mg/kg) were administered as single intraperitoneal or oral doses. Clinical chemistry and selected target organ histopathology was collected Day 2 and Day 8. High-resolution 1H-NMR spectra from urine were collected and data subjected to principal component analysis (PCA). A clear dose and time response was observed in the PCA analysis of the urine spectra with changes in agreement with clinical chemistry parameters in high-dose groups. In low-dose treated CP animals, PCA demonstrated effects at low doses which were not evident by clinical chemistry. PCA also demonstrated a time course of response, with early toxicity occurring followed by apparent recovery. These data demonstrate that the mouse is a viable test system for predictive toxicity screening using metabonomics technology.

973 APPLICATION OF NMR-BASED METABONOMIC TECHNOLOGY TO TOXICITY SCREENING: CHARACTERIZATION AND PREDICTION OF NEPHROTOXICITY


NMR-based metabonomics technology was applied to the characterization of tissue and mechanism specific renal toxicity. Acute nephrotoxicity was induced in male SD rats using a single dose of one of the following: vancomycin, thioamide, lead acetate, maleic acid, sodium chromate and sodium fluoride. Proton NMR spectroscopic analysis was performed on urine samples obtained at timed intervals following dosing. Data reduction methods and multivariate statistical analyses were applied to the spectral data and separate mathematical models constructed to characterize the metabolic effects and biomarkers of each toxin. These models were combined to form a simple expert system for classifying nephrotoxicity. Extension of this preliminary expert system will be of obvious benefit for lead compound selection in the pharmaceutical industry.

974 MULTIVARIATE STATISTICAL PRE-TREATMENT OF NMR-BASED METABONOMIC DATA TO REMOVE INHOMOGENEOUS PHYSIOLOGICAL VARIATION: CONFOUNDOING TOXICITY PATTERNS


Proton NMR-based metabonomics is a well-established technique used to analyze and interpret complex multiparametric metabolic data and has a wide number of applications in the development of pharmaceuticals. However, interpretation of biological data can be obscured by extraneous variation such as physiological variation or confounding toxicity related changes. Here we have shown the novel application of a data filtering method, orthogonal signal correction (OSC), to fluid NMR data to eliminate in vivo physiological variation and confounding toxicity patterns in two independent studies. The removal of this orthogonal variation exposed features of interest in the NMR data and facilitated biological interpretation and identification of biomarkers of toxicity. Furthermore, analysis of the orthogonal variation provided an explanation of the systematic spectral changes responsible for confounding the relevant toxicity patterns.

975 RENAL ORGANIC ANION TRANSPORTER IS A MECHANISM FOR THE RENAL UPTAKE OF MERCURIC CONJUGATES OF CYSTEINE HOMOLOGS.

A. G. Aslamkhani, Y. H. Han, J. B. Pritchard, D. W. Barbies and R. K. Zalups. 'Pharmacology and Chemistry, NIEHS, Research Triangle Park, NC, "Biology, GA State Univ, Atlanta, GA and "Basic Medical Sciences, Mercer University School of Medicine, Macon, GA.

The role of the human organic anion transporter subtype 1 (OAT1) in the uptake of mercuic conjugates of cysteine (CYM), N-acetylcysteine (NAC) or GSH was studied in a MDCKcell line stably transfected with the open reading frame sequence of the OAT1 transporter. Uptake of p-aminohippurate (PAH), which is a high-affinity substrate for OAT1, by the transfected cells was 20 times greater than that in the non-transfected cells. Mercuic conjugates, especially of CYS and NAC were significantly more toxic in OAT1-transfected cells than in non-transfected cells. The toxic effects of the mercuic conjugates of CYS were prevented by co-exposure to penicillol (a competitive inhibitor of OAT1) and partially prevented by co-exposure to Penicillol. Toxicity was also cis-inhibited by the OAT1 exchangeable dichromate alpha-ketoglutarate glutamate, and adipate. However, succinate, a non-exchangeable dichromate, did not cis-inhibit induction of toxic effects. Compared with the toxicity induced by unbound mercuic ions, which occurred steadily over a 24-hour time period, no apparent toxic effects were detected with mercuic conjugates of NAC until ten hours of exposure had elapsed. Uptake studies confirmed a direct relationship between uptake of mercury and the toxic effects elicited. Penicillol, PAH and dichromate competitively inhibited the uptake of mercuic conjugates of CYS or NAC. These findings are the first real line of mechanistic evidence showing that OAT1 transports mercuic conjugates CYS and NAC, which are species of mercury taken up in vivo and in vitro by proximal tubular epithelial cells, which are the main targets of inorganic mercury in the kidneys.

976 INORGANIC MERCURY TOXICITY AND TRANSPORT IN DISTAL SEGMENTS OF THE RABBIT NEPHRON

D. W. Barbies and R. K. Zalups. 'Biology, Georgia State University, Atlanta, GA and "Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA.

Luminal transport and acute toxicity of inorganic mercury in selected segments of the distal region of the rabbit nephron were studied using the isolated perfused tubule technique. Segments studied were the cortical thick ascending limb (cTAL)
and the cortical collection duct (cCD). Both segments were relatively resistant to the toxic effects of luminal inorganic mercury (Hg, 9.3 μM for the cCD and 20 μM for cTAL) when compared to its acute toxic effects on the proximal tubule, which are prominent cellular uptake of vital dye, cellular swelling, marked luminal membrane blebbing and a great increase in the leakiness of the tight junctions. The cCD showed no acute toxic effects from luminal mercury while the cTAL was sensitive to the presence of the luminal inorganic mercury with some slight blebbing of the luminal membrane and an increase in the leakiness of the tight junctions, but there was no cellular swelling nor cellular uptake of the vital dye. The transport of luminal inorganic mercury was measured by its disappearance rate from the lumen (JD, mmol min⁻¹ (mm tubular length)⁻¹), its appearance rate in the bath (JA, mmol min⁻¹ (mm tubular length)⁻¹) and by cellular accumulation (dmol (mm tubular length)⁻¹). The JD was 33.5 ± 2.8 and 81.6 ± 5.9 for the cCD and cTAL, respectively, while the JA was 9.3 ± 2.1 and 70.0 ± 17.4 for the cCD and cTAL respectively. The asymmetry of the JD and JA within each segment may be due to cellular uptake of the mercury, which was 1.342 ± 0.347 and 1.526 ± 0.164 in the cCD and cTAL respectively. For both the cCD and cTAL approximately 80% of the cellular accumulated mercury was associated with the tubular structure and 20% with the cytoplasm. Hence, the cCD and the cTAL can transport inorganic mercury from the luminal fluid. A significant fraction of the accumulated mercury in the cell may be due to non-specific binding. Also, these segments are relatively resistant to the acute toxic effects of inorganic mercury.

MERCURICION (Hg⁺) INCREASES THE SENSITIVITY OF KIDNEY CELLS TO APOPTOSIS BY INHIBITING NUCLEAR FACTOR-κB (NF-κB) ACTIVATION

F. J. Dieguer-Asuna, P. L. Simmons, M. E. Ellis, J. V. Kuhlheicke and J. S. Woods, Department of Environmental Health, University of Washington, Seattle, WA.

NF-κB is a thiold-dependent transcriptional factor that promotes cell survival and protects from apoptotic stimuli. Recently, we reported (TAP 173:176-187, 2001) that Hg⁺, one of the strongest thiol-binding agents known, impaired NFκB activation and transcriptional activity in normal rat kidney epithelial (NRK52E) cells at low concentrations (< 20 μM) by binding to specific reduced thiold moieties in the NFκB activation pathway. Since NFκB prevents apoptosis, we hypothesized that attenuation of NFκB activation by Hg⁺ may increase the sensitivity of kidney cells to apoptotic agents to which kidney cells are otherwise resistant because of their NFκB-activating capacity. In untreated cells, < 2% of Hg⁺ were apoptotic when evaluated by DNA fragmentation (TUNEL) or flow cytometric DNA profile analyses. Hg⁺ (5 μM) treatment for 24 hrs increased this proportion by 1.5- to 2-fold. Neither lipopolysaccharide (LPS) (1 μg/ml) nor tumor necrosis factor-α (TNF-α) (100 U/ml), known inducers of NFκB, altered the proportion of apoptotic cells, compared with untreated controls. However, when LPS or TNF were given following Hg⁺ treatment (5 μM for 30 mins.), the proportion of cells undergoing apoptosis was 22 hrs increased by 4- to 6-fold over that seen following LPS or TNF alone. In contrast, Hg⁺ pretreatment did not increase the amount of apoptosis caused by apoptosis-inducers that do not activate NFκB (staurosporine, Fas ligand). These findings support the view that Hg⁺ exerts its pro-apoptotic effect by inhibiting NFκB activity in a manner that is independent of its pro-apoptotic effects. The findings suggest that inhibition of NFκB activation may define a novel molecular mechanism by which Hg⁺ toxicity is initiated in kidney cells. Supported by ES04606 and ES07033 from NIH.

DIFFERENTIAL SENSITIVITY TO CHEMICALLY INDUCED INJURY IN PRIMARY CULTURES OF RENAL EPITHELIAL CELLS FROM CONTROL AND UNINEPHRECTOMIZED RATS

R. K. Zaluski 1, D. A. Purt 1 and L. H. Lash 1, Department of Medical Science, Mercy University School of Medicine, Muncie, IN and Pharmacology, Wayne State University School of Medicine, Detroit, MI.

Primary cultures of renal proximal tubular (PT) and distal tubular (DT) cells from rats that had undergone uninephrectomy and compensatory renal growth (NXP) retain changes in morphology, enzyme and transport activities, and mitochondrial function that occur in kidneys of NXP rats. Acute cytotoxicity of tert-butyl hydroperoxide (tBHP; 0.1, 0.5, 1, 5, 10 mM) was determined by measurement of lactate dehydrogenase (LDH) release and primary cultures of renal PT and DT cells from control and NXP rats showed increased rates of death of both cell types from NXP rats was greater than that in corresponding cells from control rats (as LDH release = 33.6% vs. 53.7% for PT cells from control and NXP; 10.8% vs. 62.1% for DT cells from control and NXP). tBHP-induced malondialdehyde formation was about 2- to 4-fold greater in PT cells from NXP rats than in cells from control rats. Similarly, tBHP and KCN (0.1-1 mM) a mitochondrial toxicant both induces significantly greater cell death in confluent primary cultures of PT and DT cells from NXP rats than in corresponding cells from control rats. For inorganic Hg (5, 10, 50 μM), however, cytotoxic effects in cells from NXP rats were similar to those in corresponding cells from control rats at the highest lower concentrations but were markedly higher at the highest concentration. Uptake of inorganic Hg (0.1, 1, 5 μM) across the brush-border and basolateral membranes of PT and DT cells grown on filter inserts was actually lower in cells from NXP rats at the two lower doses but was higher at the highest dose as compared to measurements in cells from control rats. These results indicate a greater general sensitivity of cells from NXP rats to chemical toxicants and/or toxic inorganic Hg, however, more complex dose-response relationships exist between transport and toxicity. (Supported by NIEHS Grant ES05157, ES05980, and DK40725.)

IS MERCURY IN URINE INDICATIVE OF EXPOSURE TO LOW LEVELS OF MERCURY VAPOR?

J. S. Tsui 1, P. R. Williams, M. R. Edwards 2, K. P. Avadhanyam 2 and D. P. Paustenbach 1, 1Exponent, Bellevue, WA and 2Exponent, Menlo Park, CA.

Exposure to elemental mercury has received increased attention as a result of ubiquitous use, improved analytical detection, and awareness of health effects. Although urinary mercury levels are considered the best predictor of mercury vapor exposures, a lack of relationship has been reported between airborne and urinary mercury levels at low air levels (<0.50 μg/m³). We reviewed the literature on the correlation of mercury in air and urine to evaluated whether airborne mercury exposures at health-based action levels (e.g., 1 μg/m³) are measurable in urine above background. A meta-analysis was conducted of ten studies reporting paired air and urine mercury data (149 samples total) and meeting specified criteria for data quality and sufficiency. The log-transformation of data set showed a strong correlation between mercury in air and in urine (r=0.77), although the relationship was best fit by a series of parallel lines with different intercepts for each study (R²=0.81). Predicted ratios of air to urine mercury levels at 50 μg/m³ air concentration ranged from 1:1 to 1.5:1. Toward the lower end of the dataset (i.e., 10 μg/m³), predicted urinary mercury levels encompassed two distinct ranges; values on the order of 20 μg/l and 30-60 μg/l. Extrapolation to 1 μg/m³ resulted in predicted urinary levels of 4-5 and 6-15 μg/l. Higher predicted urine mercury levels were associated with use of static air air samplers by some studies, rather than more accurate personal air samplers. Urinary mercury predictions based primarily on personal air samplers at 1 and 10 μg/m³ are consistent with reported mean (4 μg/l) and upper-bound (20 μg/l) background levels, respectively. Thus, although mercury levels in air and urine are correlated below 50 μg/m³, the impact of mercury in air below 10 μg/m³ is likely indistinguishable from background urinary mercury levels.

MERCURIC CHLORIDE SUPPRESSES CYCLOXOGENASE-2 EXPRESSION AND PROSTAGLANDIN PRODUCTION IN A HUMAN MONOCYTOCYTIC CELL LINE

W. C. Glasgow and R. K. Zaluski. Basic Medical Sciences, Mercer University School of Medicine, Macon, GA.

We examined the effects of a brief treatment of low-dose mercuric chloride on the activation of arachidonic acid metabolism in the human monocytic cell line THP-1. cPLA₂ analysis revealed that LPS treatment stimulated induction of cyclooxygenase-2 expression and production of prostaglandin E₂ (a major arachidonate metabolite) in these cells. Unstimulated THP-1 cells express the constitutive cyclooxygenase-1 isoenzyme and a low level of cyclooxygenase-2, with a basal production of prostaglandin E₂. THP-1 cells were pre-treated with 1 microM mercuric chloride for one hour, and then were incubated with LPS (1 microg/ml) for 4 hours. Cell viability was not affected by treatment with inorganic mercury, as assessed by trypan blue exclusion. Treatment with mercuric chloride suppressed both the basal level and LPS-stimulated expression of cyclooxygenase-2, as measured by Western-blot analysis. Correspondingly, EIA analysis revealed that both basal and LPS-stimulated prostaglandin E₂ synthesis was attenuated by mercuric chloride treatment. We did not observe alteration of cyclooxygenase-1 expression. These results demonstrate an effect of mercuric chloride on inactivating the cyclooxygenase pathway in activated monocytes/macrophages. Modulation of this pathway by inorganic mercury suggests that part of the adverse effects of exposure to low levels of this metal may be suppression of inflammatory and immune responses.

EFFECTS OF METHYLMERCURY EXPOSURE ON THE IMMUNE AND nervous system RESPONSES OF CBA/J MICE TO A CHRONIC TOXOPHOLASMA GONDII INFECTION

M. D. King, M. Ehrlich and D. S. Lindsay, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Previous studies from our laboratory suggest that exposure to methyl mercury (MeHg) does not increase the susceptibility to acute toxoplasmosis in CBA/J mice. Therefore, we investigated endpoints associated with immunotoxicity and neuro-
toxicity in 6-week-old, female CBA/J mice exposed to both MeHg and T. gondii during a chronic T. gondii infection. Mice were fed 25 T. gondii tissue cysts and dosed six weeks later with 20 mg/kg of MeHg. There were significant differences (P < 0.05) within the T cell subpopulation ratios as indicated by flow cytometric analysis. MeHg increased CD4+ CD8+ T cells and subpopulations with the CD4+ T cell subpopulation ratios were increased (P<0.05). MeHg had no effect on the CD4+ CD8+ T cell or non-T cell subpopulations. Groups without T. gondii infection showed a decrease in CD8+ T cells when compared to groups with T. gondii infection in the CD4+ T cells between infected or non-infected mice. There was a significant (P<0.05) increase in brain tissue cyst counts within the group exposed to both MeHg and T. gondii (16 ± 4, mean ± SE, n=7) versus T. gondii alone (4 ± 1, n=8). These data indicate that concurrent exposure to both MeHg and T. gondii has notable effects, especially on the immune system. (Supported by N1H Grant F33GM20301.)

2015 ORGANIC MERCURY (HgC12) DECREASES ADIPOGENESIS AND CAUSES INSULIN RESISTANCE IN 3T3-L1 ADIPOCYTES.

E. A. Kercher and D. M. Barnes. Animal Sciences; Molecular and Environmental Toxicology Center, University of Wisconsin - Madison, Madison, WI.

Many aspects of the differentiation process of the 3T3-L1 preadipocyte fibroblast cell line have been determined and can be used as a model to study the effects of toxins on adipogenesis. Mercury (Hg) is a prevalent and mobile toxin in our environment that has been shown to increase the glucose uptake of mature adipocytes. In this report, we examine the effects of Hg on adipocyte differentiation and insulin responsiveness. 3T3-L1 fibroblasts were differentiated in the presence or absence of 1-10 μM Hg. The presence of Hg decreased the number of cells differentiating to adipocytes, decreased their lipid content, and decreased expression of PPARα. Lipid content of cells differentiated in the presence of 5 or 10 μM Hg decreased by 22 and 21% respectively. Hg reduced the number of differentiated adipocytes by 14, 36 and 55% for 5, 10 and 20 μM Hg respectively. PPARα expression decreased 60% in the presence of 5 and 10 μM Hg. Another physiological consequence of Hg exposure during differentiation was a reduction of insulin mediated glucose uptake. Cells differentiated in the presence of 1, 5, and 10 μM Hg decreased their insulin mediated glucose uptake by 25, 57, and 62% respectively. These results show that low levels of Hg can disrupt adipocyte differentiation and alter their subsequent physiological responses to a hormonal stimulus.

2015 MERCURY CONTAMINATION IN INTERNAL ORGANS OF CETACEANS MARKETED FOR HUMAN CONSUMPTION.

T. Endo1, K. Haraguchi1 and M. Sakata1. 1Pharmaceutical Science, Health Sciences University of Hokkaido, ithi2hini-Itieus, Hokkaido, Japan and 2Daichi College of Pharmaceutical Sciences, Minami-ku, Fukuoka, Japan.

The internal organs such as liver, kidney, lung, and small intestine of toothed whales and dolphins have been traditionally eaten in the limited area of Japan. A mixture of these organs is sold as boiled products. We surveyed Hg contamination in the internal organs of cetaceans sold in Japanese market for human consumption. The averages with SD of total mercury (T-Hg) were 357 ± 520 (0.195–1980, n=27) ppb in liver, 40.5 ± 485 (7.85–95.1, n=15) ppb in kidney and 42.8 ± 43.8 (2.10–75.6, n=23) ppb in lung. The proportional limit of T-Hg in marine products set by Japanese Ministry of Health and Welfare is 0.4 ppm. The higher of T-Hg detected in the liver was about 5000 times higher than their limit. suggesting acute intoxication of Hg even by a single consumption. A high correlation was observed between T-Hg and Se concentrations; the molar ratio of T-Hg/Se in these organs was slightly higher than 1. Furthermore, a significant correlation was observed between Zn and Cu concentrations in liver and lung, suggesting the induction of metallothionein (MT). Probably, the formation of Hg-Se complex and the induction of MT should decrease the toxicity of Hg for cetaceans and allow tremendous accumulation of Hg in their livers. Supported by IFAW.

2015 GADOLINIUM PRETREATMENT PREVENTS CADMIUM-INDUCED LIVER DAMAGE IN BOTH WILD-TYPE AND METALLOTHIONEIN-NULL MICE.

E. B. Harstad and D. C. Klaassen. University of Kansas Medical Center, Kansas City, KS.

The heavy metal cadmium (Cd) causes hepatotoxicity upon acute administration. Kupffer cells, the resident macrophages of the liver, have been implicated in the mechanism of Cd-induced hepatotoxicity, as suppressing Kupffer cells with gadolinium chloride (GdCl3) prevents Cd-induced hepatotoxicity. However, GdCl3 may also induce the Cd-binding protein, metallothionein (MT). Therefore, a study was conducted to determine whether GdCl3 prevents Cd-induced hepatotoxicity via the induction of MT or depletion of Kupffer cells. First, Hepatic MT and Kupffer cell counts were analyzed 24 h after administration of saline or 10, 30, or 60 mg GdCl3/kg to wild-type (WT) mice. Second, WT and MT-null mice were pretreated with saline or 10, 30, or 60 mg GdCl3/kg 24 h prior to a hepatotoxic dose of Cd (2.5 mg Cd/kg) to examine whether the hepatoprotective effects of

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GdCl₃ are related to the observed induction of MT or Kupffer cell activation. Blood and livers were removed 16 h after Cd administration and analyzed for hepatotoxicity as well as MT and Kupffer cell content. GdCl₃ treatment alone induced MT in a dose-dependent manner and did not affect hepatic non-protein sulfhydryl content. Cd-induced hepatotoxicity was prevented in both WT and MT-null mice that were pretreated with 30 or 60 mg GdCl₃/kg, demonstrating that MT induction is not required for the hepato-protective effects of GdCl₃. Hepatic Cd content was not decreased by GdCl₃, demonstrating that GdCl₃ did not alter Cd distribution to the liver. Furthermore, Kupffer cells were depleted at all doses of GdCl₃, whereas hepatoprotection was only observed at doses of 30 and 60 mg, but not 10 GdCl₃/kg. This did not conclusively rule out Kupffer cells in the mechanism of Cd-induced hepatotoxicity, but it does suggest that GdCl₃ exerts hepatoprotective effects on the liver in addition to depleting Kupffer cells. In summary, these data rule out MT induction and suggest a limited role for Kupffer cells as mechanisms of GdCl₃-induced protection from Cd-induced hepatotoxicity. (Supported by NIH grants ES-01142 and ES-67079)

987 MECHANISM OF RESISTANCE TO CADMIUM TOXICITY IN MALE WISTAR-IMAMICHI RATS.

H. Shimada¹, M. Nagano¹, A. Yasutake¹ and Y. Imamura¹. ¹Faculty of Education, Kumamoto University, Kumamoto, Japan, ²National Institute for Minamata Disease, Kumamoto, Japan and ³Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

It has been shown that the toxic effect of cadmium (Cd) can be varied with species, strain and sex in experimental animals. For these reasons, one of the key elements is thought to be metallothionein (MT) that plays a critical role in detoxification of Cd. However, the detailed mechanism for these variations of Cd toxicity remains to be elucidated. In the present study, we compared Cd-induced mortality in four strains of male rats. In the case of Fischer, Wistar and Sprague-Dawley strains, a single injection of 5 mg Cd/kg proved very toxic and induced 80-100% mortality within 2 days. On the other hand, in Wistar-Imamichi strain, the Cd treatment proved nontoxic during 7 days. Next, we attempted to analyze the inheritance pattern of Cd resistance, using resistant (Wistar-Imamichi) and sensitive (Fischer) inbred rats to the metal. The resistance to Cd toxicity was also observed in male rats of first filial (F1) generation (male Fischer x female Wistar-Imamichi). In order to establish whether the resistance to Cd toxicity in male Wistar-Imamichi rats is an- droneg- and dependent, the effect of testisectomy on Cd-induced mortality was examined. However, testesctomy had no effect on the resistance to Cd toxicity in male Wistar-Imamichi rats, suggesting the resistance is independent of the regulation by androgens. Furthermore, we determined GSH and MT levels in several tissues of resistant (Wistar-Imamichi) and sensitive (Fischer) rats. Pronounced difference of GSH and MT levels in these two strains was not observed in the control or Cd-treated rats. Therefore, it is possible to assume that endogenous substance(s) other than GSH and MT is involved in the resistance to Cd toxicity found in male Wistar-Imamichi rats. Further studies are in progress to elucidate the mechanism of the resistance to Cd toxicity in male Wistar-Imamichi rats.

988 INDUCTION OF METALLOTHIONEIN BY CADMIUM IS ASSOCIATED WITH P53 AND APOPTOSIS IN HUMAN EPITHELIAL BREAST CANCER CELLS.

L. Fan and M. G. Chetian. Pathology, University of Western Ontario, London, ON, Canada.

Although the function of metallothionein (MT), a metal-binding protein, remains unclear, previous studies in human tumors and cellular differentiation have suggested a pro-apoptotic function. Since the tumor suppressor protein p53 plays an important role in apoptosis, the present study was undertaken to investigate any potential relationship between MT and p53 using human breast cancer epithelial cell lines differing in p53 and estrogen-receptor (ER) status. Cells were treated with 10 μM GdCl₃ for 24 h, and MT protein and mRNA levels were measured by Western blot analysis and RT-PCR. MCF7 cells (p53⁻, ER⁺) showed a much greater induction in MT protein and mRNA expression as compared to cell lines which are p53⁻ and ER⁻ or weakly positive for ER (ERA). MT localization was determined by immunohistochemical staining. Prior to Cd treatment, MCF7 and HCC1806 (p53⁻, ER⁻) cells both showed mainly cytoplasmic staining. Following treatment, MCF7 cells showed intense nuclear and cytoplasmic staining while HCC1806 cells showed staining in the cytoplasm and weak staining in the nucleus. The TUNEL technique was used to study Cd-induced apoptosis. It was found that 10-40 μM CdCl₂ treatments caused significant apoptosis in MCF7 cells, particularly at 6, 8 and 48 h, but not in MDA-MB-231 cells (p53⁻, ER⁻). The data demonstrate that the induction of MT expression is higher in p53⁻ cells than MCF7 or p53⁺ cells. It also suggests that Cd-induced apoptosis may occur mainly in p53⁻ cells and could be a p53-dependent pathway. Since these cells can induce MT activity after exposure to Cd, this may explain the pro- and anti-apoptotic properties of Cd salts at different concentrations. Additional studies after transfection of p53 gene into p53⁻ epithelial cells or inactivation of p53 gene in p53⁺ cells may explain the mechanisms involved in MT induction and changes in apoptosis. (Supported by grants from CIHR and NSERC)

989 CADMIUM INDUCED APOPTOSIS IN HUMAN PROXIMAL TUBULE CELLS AND ITS DERIVED CELL LINE, HK-2.

S. Somji, H. G. Garrett, M. A. Sens and D. A. Sens. Urology, West Virginia University, Morgantown, WV.

Cadmium (Cd⁺²), a nephrotoxin, can cause acute and chronic toxicity in man due to its slow accumulation in the proximal tubules of the kidney. Heavy metals have been known to cause cell death by necrosis or by apoptosis. The goal of this study was to determine if Cd⁺² could induce apoptosis in the human proximal tubule (HPT) cells and its derived immortalized cell line, HK-2. For this purpose, HPT and HK-2 cells were exposed to lethal and sub-lethal concentrations of cadmium chloride for various time periods. Apoptosis was determined by observing the nuclear morphology with the nuclear binding dye, 4', 6-diamidino-2-phenylindole (DAPI), and internucleosomal DNA fragmentation. Continuous exposure of HPT cells to 27 μM cadmium chloride for 12 to 60 hr caused cell death, with few cells showing the morphological changes typical for apoptosis and little DNA fragmentation. Exposure to 45 μM cadmium chloride caused extensive cell death without any changes typical for apoptosis. HK-2 cells were more sensitive to Cd²⁺ and therefore lower doses of 4.5 and 9 μM Cd²⁺ were used which resulted in cell death with extensive chromatin condensation and DNA fragmentation. These results suggest that apoptosis is not the major mode of cell death elicited by Cd⁺² in HPT cells and is concentration dependent, whereas HK-2 cells are more susceptible to death by apoptosis.

990 INDUCTION OF APOPTOSIS IN CELLS BY CADMIUM; QUANTITATIVE NEGATIVE CORRELATION BETWEEN BASAL OR IMMUNOLABELED METALLOTHIONEINE CONCENTRATION AND APOPTOTIC RATE.

R. Shimoda¹, T. Nagamine¹, H. Takagi¹, M. Mori¹ and M. P. Wiakek¹. ¹NICI at NIES, Research Triangle Park, NC, ²First Department of Internal Medicine, Gunma University, Maebashi, Japan and ³Dept. of Health Science, Gunma University, Maebashi, Japan.

Metallothionein (MT) often reduces the adverse effects of cadmium (Cd) but how it may alter Cd-induced apoptosis is unclear. Thus, the goal of this study was to define the role of MT in Cd-induced apoptosis using cell lines with widely varying sensitivity to Cd. Toxic effects of Cd on human hepatocellular carcinoma cell lines (HepG2 and PLC/PRF5) were investigated and compared with Chang cells. These cells were cultured with 0, 5, 10, 20, 40, 80 and 120 μM of Cd for 3, 6, 12, and 24 h. Significant cytotoxicity was observed in HepG2 and PLC/PRF5 cells in time-dependent and concentration-dependent manner with LC₅₀ values of 24 μM and 13 μM, respectively. However, Chang cells were much less sensitive to Cd-induced cytotoxicity (LC₅₀ = 64 μM). Apoptotic cell death occurring at cytotoxic concentrations was demonstrated in all cell lines by DNA fragmentation with ELISA and confirmed with agarose gel electrophoresis. When MT was measured, there was a strong negative linear correlation between the rate of apoptosis induced by Cd in the cell lines and the basal levels of cellular MT concentration (r = -0.956) or Cd-induced MT levels (r = 0.975). Treating HepG2 cells with zinc (Zn) made the relatively sensitive HepG2 cell line resistant to Cd-induced apoptosis, likely due to Zn-induced MT. In fact, there was also a strong negative linear correlation (r = -0.964) between the amount of Zn-induced MT in HepG2 cells and the rate of Cd-induced apoptosis. These findings revealed that Cd-induced apoptotic cell death in various non-neuronal mammalian cell lines and induction of MT and a strong negative correlation exists between cellular MT content and the rate of apoptotic cell death induced by Cd.

991 CADMIUM INDUCES APOPTOSIS IN RAT KIDNEY PROXIMAL TUBULAR CELLS VIA THE MITOCHONDRIAL PATHWAY.

S. Ge and Z. A. Shalik. Biomedical Sciences, University of Rhode Island, Kingston, RI.

Chronic cadmium (Cd) exposure causes renal proximal tubular cell damage and mitochondria appear to be the target organelle. The purpose of this study was to determine whether in these cells Cd-induced mitochondrial damage is responsible for apoptotic cell death. Normal rat kidney proximal tubular cells (NRK-52E) were exposed to 20 μM CdCl₂ for 5 h, followed by continuous culture in the absence of Cd for up to 12 h. Formation of reactive oxygen species was measured by hydrogen
peroxide accumulation and was found to be maximum 3 h after starting the Cd exposure. Cytochrome c release from the mitochondria to the cytosol peaked between 3 and 5 h. Caspase-3 activity was markedly elevated 12 h after stopping the Cd exposure and was higher in detached cells than in attached cells. Cytochrome c release was accompanied by the induction of mitochondrial permeability transition with cytochrome c release. The use of these inhibitors also reduced caspase-3 activity. Furthermore, both cytochrome c release and caspase-3 activity were depressed in cells expressing Bcl-2. In conclusion, Cd-induced oxidative stress in rat kidney proximal tubular cell mitochondria appears to play a key role in apoptosis since generation of reactive oxygen species is followed by mitochondrial membrane permeability transition, cytochrome c release, and caspases-3 activation.

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THE NITRIC OXIDE PRODRUG, V-PYRRO/NO, CONFRONTS RESISTANCE TO ARSENIC AND CADMIUM-INDUCED CYTOTOXICITY AND APOPTOSIS IN CULTURED RAT LIVER CELLS.

W. Qi,1 R. Fuquay2 L. K. Keever2 and M. P. Wallace1 1. ILC, NCI at NIEHS, Research Triangle Park, NC and 2. ILC, NCI at Frederick, Frederick, MD.

The liver is an important target tissue of both cadmium (Cd) and arsenic (As). The compound O2- vinyl 1-(pyrrolin-1-yl)-1-diazene-1-ium-1, 2 diolate (V-PYRRO/NO) is a liver-selective nitric oxide (NO) producing produg that is metabolized by hepatic P450 enzymes to release NO in hepatocytes. NO can compete for cellular binding sites with metals. Thus, we studied the effects of V-PYRRO/NO pretreatment on Cd (as CdCl2) or As (as NaAsO2) toxicity in cultured rat liver epithelial (TRL 1215) cells. Cells were pretreated with V-PYRRO/NO (at levels up to 1000 μM for up to 24 h) then exposed to Cd or As (for an additional 24 h) and cytotoxicity (by MTS assay) or apoptosis (by DNA fragmentation ELISA) was assessed. Cd was significantly less cytotoxic in V-PYRRO/NO (1000 μM) pretreated cells (LC50 = 6.1 ± 0.6 μM) compared to control cells (LC50 = 3.5 ± 0.4 μM). Likewise, the LC50 for As was 18.1 ± 1.4 μM in control cells and 27.3 ± 2.6 μM in V-PYRRO/NO pretreated cells. TRL 1215 cells acted upon the produg to release NO, producing nitrite levels (measured by Griess assay) in the extracellular media after 24 h of exposure to 500 or 1000 μM V-PYRRO/NO measured at 870 ± 4.2 and 324 ± 14.8 μM, respectively, compared to control levels of 7.70 ± 0.46 μM. Since both Cd and As are known to induce apoptosis, the effects of V-PYRRO/NO pretreatment on Cd- or As-induced apoptosis was studied. V-PYRRO/NO pretreatment (275 μM) markedly reduced apoptotic cell death induced by Cd (5 μM) or As (15 μM). Activation of the c-Jun N-terminal kinase (JNK) pathway can be critical to apoptotic cell death and pretreatment of cells with V-PYRRO/NO suppressed JNK activation after exposure to Cd or As. Thus, the produg, V-PYRRO/NO, protects against the adverse effects of Cd or As in rat liver cells in culture, including apoptosis and the concurrent activation of the JNK pathway, apparently through generation of NO. The role of NO in prevention of Cd or As toxicity deserves further study.

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EFFECTS OF CADMIUM ON E-CADHERIN AND VE-CADHERIN/β-CATENIN IN MOUSE LUNG.

C. A. Pearson, P. C. Lamb and W. C. Prozialek, Pharmacology, Midwestern University, DeKoven Grove, IL.

Although it is well established that respiratory exposure to Cd+2 results in pulmonary edema and fibrosis, the specific mechanisms underlying these effects remain unknown. Recently, we have obtained evidence suggesting that Cd+2 produces these effects by interfering with the normal function of the Cd+2-dependent cell adhesion molecules E-cadherin and VE-cadherin. These molecules serve as important structural components of adherens junctions in alveolar epithelial cells and vascular endothelial cells respectively. In addition, the intracellular domains of the cadherins are linked to β-catenin, which under certain circumstances can be released from the junctional complexes and act as a regulator of gene expression. The objective of the present study was to characterize the effects of Cd+2 on the localization of E-cadherin, VE-cadherin and β-catenin in relation to the development of acute pulmonary injury. Male C57-1 mice were given either saline or CdCl2 (65 nmole) intratracheally administration instillation. After 24 hours, the lungs were subjected to bronchoalveolar lavage or weighed and processed for the immunohistochemical analyses and for the evaluation of cell viability with ethidium homodimer. The results showed that Cd+2 caused an increase in lung weight and in the protein content of the lavage fluid. These effects were accompanied by a pronounced decrease in the amount of E-cadherin in the epithelial cells of the alveoli and small bronchioles and of VE-cadherin in the vascular endothelial cells. In addition, the pattern of β-catenin labeling changed from co-localization with the cadherins at the plasma membrane to diffuse labeling throughout the cytoplasm. Additionally, a thin band of β-catenin labeling was observed at the cell nuclei. Assessment of cell membrane integrity with ethidium homodimer showed no evidence of severe injury or death of the alveolar epithelial or vascular endothelial cells. These findings indicate that the E-cadherin and VE-cadherin/β-catenin complexes may be important early targets of Cd+2 toxicity in the lung. Supported by NIH Grant R01 ES06478.

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EFFECTS OF P-GLYCOPROTEIN INHIBITORS ON CADMIUM ACCUMULATION IN CULTURED EPITHELIAL CELLS, OK, LLC-PK, AND CACO-2.


The effect of p-glycoprotein (P-gp) inhibitors on cadmium (Cd) accumulation in cultured epithelial cells, OK, LLC-PK, and Caco-2 was investigated. These cells were pre-incubated for 30 min with a typical P-gp inhibitor such as verapamil, dilzem, nimodipine, vinblastine or cyclosporin A before the incubation with 1 μM CdCl2 for 15 min. Pre-incubation with each P-gp inhibitor increased Cd accumulation markedly in OK and LLC-PK, cells and marginally in Caco-2 cells. Similarly, treatment of these cells with U122, a P-gp monoclonal antibody, increased Cd accumulation significantly in OK and LLC-PK, cells and marginally in Caco-2 cells. Thus, the pre-treatment of cells with P-gp inhibitor appears to increase Cd accumulation as a result of the inhibition of Cd efflux via P-gp. To confirm Cd efflux via P-gp, Cd accumulation in P-gp-overexpressed cells (LLC-GAS-COL150 cells) was compared with that in parental LLC-PK, cells. LLC-GAS-COL150 cells accumulated less Cd than did LLC-PK, cells, and the effects of the P-gp inhibitors and U122 on Cd accumulation were profoundly observed in LLC-GAS-COL150 cells. These results suggest that P-gp in OK, 15, LLC-PK, and Caco-2 cells appears to act as an efflux pump of Cd, decreasing cellular Cd accumulation.

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HEAT SHOCK RESPONSE IN THE HUMAN URINARY UROTHELIAL CELL LINE, UROtsa, EXPOSED TO CADMIUM AND ARSENITE.


Urology, West Virginia University, Morgantown, WV, Pathology, West Virginia University, Morgantown, WV and Genetics and Developmental Biology, West Virginia University, Morgantown, WV.

The heat shock response of specific tissues to acute versus chronic exposure to toxic substances has not been addressed in the literature. We therefore wanted to determine if the heat shock response was a transient or persistent phenomena in UROtsa cells acutely and chronically exposed to chemical stress. UROtsa cells are immortalized human urothelial cells that exhibit several features unique to normal urothelium. These cells were treated with sodium arsenite or cadmium chloride and mRNA and protein levels were detected by RT-PCR or western blot respectively. Acute exposure to 100 μM sodium arsenite or 53.4 μM cadmium chloride for 4 hours increased the levels of heat shock protein (hsp) 60, 70A, 70B and 70C mRNA and hsp 70 protein. There was no change in the level of hsp 27 mRNA and protein, and hsp 60 protein following acute exposure. Chronic exposure to 1.5 or 5 μM Sodium arsenite for 16 days had little effect on hsp 27, 60, 70A, 70B or 70C mRNA levels, but hsp 70 protein was consistently present in cells treated with 5 and 9 μM sodium arsenite. Similarly, chronic exposure to various doses of cadmium chloride also demonstrated no obvious induction of hsp 27, 60, 70A or 70B genes. There was an increase in levels of hsp 70C gene expression in UROtsa cells chronically exposed to 5 and 9 μM cadmium, but the mRNA and protein levels decreased after 4 days with protein levels undetected after 7 days. These findings suggest that the heat shock response can be both transient and persistent, and that the duration of the response may be dependent upon the chemical treatment.

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SIX-MONTH DRINKING WATER STUDY TO ESTABLISH A LOEL FOR CADMIUM TOXICITY.

G. Wang, M. H. Whitaker, M. Lipsky, X. Chen and B. A. Fowler, Epidemiology and Preventive Medicine, The University of Maryland, Baltimore, MD.

A lowest observed effect level (LOEL) associated with the oral ingestion of cadmium was investigated in a six month drinking water study containing 10, 25, and 50 ppm cadmium (as cadmium chloride) for durations of 30, 90, and 180 days. Signs of toxicity were evident at each timepoint, as evidenced by increased total urinary protein/24 hours and decreased body weight changes. At all three cadmium concentrations at days 90 and 180, urinary protein levels were greater than that of the control value. Among the three dose groups at days 30 and 180, body weight changes were decreased (65-89%) compared to control. Text animals adapted/comensated to the ingestion of cadmium over time for some parameters, and early increases in blood ALAD levels (156%-172% of control), and early decreases in
ANEMIA IN CHRONIC CADMIUM INTOXICATION IS INDUCED BY THE DESTRUCTION OF ERYTHROPOIETIN-PRODUCING CELLS IN KIDNEYS.

H. Horiguchi1,2, E. Oguma2,3 and F. Kayama1,2.
1Health Science, Jichi Medical School, Minamikawachi-machi, Japan and 2CREST, JST, Kagoshima, Japan. Sponsor: T. Yashida.

Long term exposure to cadmium (Cd) induces renal tubular dysfunction and anemia in mice. We have therefore tried to elucidate the cell types involved in the process. However, plasma levels of one of the major hematopoietic growth factors, which is produced mainly from the interstitial cells of kidney, do not increase in response to anemia in man and patients with chronic Cd intoxication. Thus, it is suggested that Cd induces anemia through disturbance of Epo production by target cells. Epo-producing cells in the renal interstitial tissues. In order to investigate the local relationship between Cd-target cells and Epo-producing cells in kidneys, we performed in situ hybridization using Epo cDNA probe as well as hematoxin-eosin staining of the renal tissue sections of rats which were injected with Cd for 8 months. Epo mRNA was expressed in renal tubular cells, but these cells were not involved in the interstitial tissues. The results indicate that anemia with hydropoiesis of Epo-producing cells in kidneys is induced by the destruction of Epo-producing cells in kidneys; i.e. renal tubular cells.

INTESTINAL ABSORPTION OF CADMIUM IS ASSOCIATED WITH DIVERVAL METAL TRANSPORTER 1 IN RATS.

J. D. Park1,2, N. J. Cherrington3 and C. D. Klaassen1.
1Pharmacology Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS and 3Chung-Ang University, Seoul, South Korea.

The intestinal absorption of cadmium (Cd) increases when body iron (Fe) stores are depleted. The depletion of Fe uptake-regulates the expression of Divalent Metal Transporter 1 (DMT1), which is located in the apical membrane of enterocytes lining the small intestine. DMT1 has been shown to transport Fe and other divalent metal ions in vitro. However, it is not known whether DMT1 mediates the intestinal absorption of Cd. To investigate DMT1 involvement in Cd absorption, rats were fed a diet for 4 weeks either deficient in Fe (Fe-diet, 2-6 mg Fe/kg) or supplemented with Fe (Fe-sup diet, 120 mg Fe/kg), followed by a single oral administration of 100 mg CdCl2. Body weight, hematocrit, and tissue Cd concentration were determined at 48 hr after Cd administration. Also, DMT1 mRNA levels were quantified in duodenum, kidney, and liver by the branched DNA signal amplification method. Anorexia and the intestinal exhibited a reduced body weight gain, depletion of body iron, and Fe deficiency anemia. Tissue Cd concentration was significantly higher in Fe-deficient fed rats than in Fe-sup diet fed rats especially in the duodenum. The amount of Cd retained in the body was 10-fold higher in rats fed the Fe-deficient diet than in Fe-sup diet fed rats. DMT1 mRNA was highly expressed in duodenum and was 15-fold higher in the Fe-deficient diet. The levels of DMT1 mRNA were significantly lower in kidney and liver than in duodenum, but were 50 and 40 percent higher, respectively, in rats fed the Fe-deficient diet than in rats fed the Fe-sup diet. These findings suggest that functional DMT1 protein is likely up-regulated in small intestine at the mRNA level by body iron depletion and increases Cd uptake from the gut. This study was supported by NIH grants ES-01142 and ES-07079.

SUBACUTE HEMATOLOGICAL RESPONSES OF THE YUCATAN SAILFISH MOLLY (POECILIA VELIFERA) EXPOSED TO CADMIUM CHLORIDE.

S. M. Rustnak1, J. Fourrie1 and W. R. Harley1.
1School of Public Health and Tropical Medicine, Tulane University Health Sciences Center, New Orleans, LA and 2Gulf Ecology Division, NHEERL, USEPA, Gulf Breeze, FL. Sponsor: C. Miller.

This study focuses on the subacute hematological responses of the Yucatan sailfish molly, Poecilia velifera, exposed to cadmium chloride. Previous studies in other teleosts and mammals have suggested that exposure to cadmium chloride results in a leukocytic response. Fish were exposed to nominal concentrations of 9, 5, 0.9, and 0.01 mg/L of cadmium chloride for seven days (n=100) in static exposure chambers. At the end of exposure period, blood smears were taken from each fish, fixed in methanol, and stained with Giemsa. Differential and total blood cell counts were determined and used to find the absolute blood cell count. Peripheral blood leukocytes, which were not characterized in this study, were observed and described. Lymphocytes, large and small monocytes, and immature granulocytes were observed. Basophils and neutrophils were not observed in this species. Preliminary findings indicate a significant increase in the total leukocyte count as well as the absolute eosinophil count in fish exposed to cadmium chloride compared to the control group (n=20). The potential use of the Yucatan sailfish molly as an environmental model for adverse hematological/immunological effects is discussed.

ACTIVATION OF AP-1 DNA BINDING IN MAMMALS TOXICITY IN PC-12 CELLS.

G. T. Rance1, E. Ghozli2, and P. G. Gunsalas2.
1Biology, Texas Southern University, Houston, TX and 2Pharmacology & Toxicology, Wright State University, Dayton, OH.

Occupational and environmental exposure to manganese (Mn) is an increasing problem. It manifests neuronal degeneration characterized by dyskinesia resembling Parkinson's disease. The study was performed to test the hypothesis whether exposure to Mn2+ alters cellular physiology through intracellular signaling mechanisms associated with toxicity in neuronal cell lines. Using rat catecholaminergic neuropharmacology (PC12) cells, various concentrations of Mn2+ (0-100 mM) were exposed and their viability was measured with MTT assay. Exposure of Mn2+ to PC12 cells reduced cell viability to a dose dependent manner compared to the controls. Since transcription factors have been shown to play an essential role in oxidative stress related toxicity, we measured changes in DNA binding activity of activator protein-1 (AP-1) by electrophoretic mobility shift assay (EMSA) following Mn2+ exposure in PC12 cells. Mn2+ (1-10 mM) produced eight-fold activation of AP-1 DNA binding activity. This significant increase was observed within 60 min period after exposure. Besides activating AP-1, Mn2+ also activated N terminal c-Jun kinase suggesting that a JNK pathway is involved, which may in turn activate AP-1 via c-Jun phosphorylation. This study clearly suggests that induction of signaling factor AP-1 during Mn2+ exposure may have a significant cytotoxic effect on neuronal function. This work was supported by R21 grant RR03504.

MANGANESE OXIDATION STATE AND CELLULAR TOXICITY.

S. H. Reaney1,2, C. Kwok-Ubri3 and D. R. Smith1.
1Chemistry and Biochemistry, UCSC; Santa Cruz, CA and 2Environmental Toxicology, UCSC; Santa Cruz, CA.

BACKGROUND: Chronic elevated manganous (Mn) exposure has been associated with neurological disorders. The introduction of the anti-oxidant agent MMT to gasoline has lead to concern about the potential for increased Mn neurotoxicity. The combustion products of MMT may increase exposure to a mixture of Mn oxidation states (MnII, MnIII) and MnIV), which may possess different relative toxicity. However, little reliable data exist on the biological handling of MnII since Mn is from environmental exposures, or on the relative toxicity of these oxidation states in biological systems. This lack of data is due in part to the challenges associated with evaluating Mn oxidation state in vivo. Therefore, the goal of this research was to elucidate the relative toxicity of MnII and MnIII in a cell model. Total 8-isoprostanes were measured as a marker of cellular toxicity in a dopaminergic cell line (PC12). Cells were exposed to MnII and MnIII in vitro culture medium (0 - 250uM). The validity of the MnII and MnIII exposures was verified by EPR spectroscopy and ICP-MS. Since addition of the pro-oxidant MnIII directly to the cell culture system can damage cells via processes that may not be relevant to effects of intra-cellular processes, MnII and MnIII were added to medium and incubated prior to exposing the cells. Cells were exposed for 18 hours. Results show that 8-isoprostane levels were significantly (2x-fold) increased following 250uM MnIII exposure, relative to controls. Exposures to MnII at these levels did not result in measurable increases in 8-isoprostanes. These results indicate that MnII is more toxic to cells than MnIII via a mechanism that involves increased oxidative stress and lipid peroxidation.

THE EFFECT OF MANGANESE EXPOSURE ON IRON HOMEOSTASIS IN CULTURED PC-12 CELLS.

C. L. Kwok-Ubri, S. H. Reaney and D. R. Smith.
Environmental Toxicology, University of CA; Santa Cruz, Santa Cruz, CA.

Manganese (Mn) is a well-known neurotoxicant; however, its mechanism(s) of action remains elusive. We hypothesize that one factor contributing to Mn neurotoxic effects is Mn-induced disruptions in intracellular iron (Fe) homeostasis as a
consequence of alterations to the post-transcriptional regulation of Fe responsive proteins. To investigate this hypothesis, undifferentiated PC-12 cells were cultured in 0.5% serum containing RPMI medium supplemented with 1-200 μM Mn as MnCl₂ for 24-72 hours. Intracellular Mn concentrations significantly increased over the culture period, reaching concentrations up to 50 times higher than that measured in control cells. This increase in intracellular Mn concentrations was correlated with changes in the intracellular Fe pool. In addition, the protein levels of the Fe-responsive proteins ferritin, transferrin receptor, and DMT-1 were also altered as a result of Mn exposure. The pattern in protein expression changes produced by exposure to Mn was similar to that observed for PC-12 cells cultured in serum-free medium, leading us to model cellular Fe deficiency. These data demonstrate that Mn has the ability to disrupt not only the expression of Fe-responsive proteins, but also the intracellular availability of Fe, suggesting that Mn-induced changes in cellular Fe homeostasis may contribute to the neurotoxic effects of Mn.

1003 THE INFUX OF MANGANESE, MANGANESE CITRATE, AND MANGANESE TRANSFERRIN AT THE BLOOD-BRAIN BARRIER IS CARRIER-MEDIATED.

J. S. Crossgrove, J. D. Allen, B. B. Bukwadeka, S. S. Rhineheimer and R. A. Yokel, University of Kentucky, Lexington, KY; Texas Tech University Health Sciences Center, Amarillo, TX and University of Louisville, Louisville, KY.

Manganese (Mn) is required for normal brain function, yet at high levels it can cause neurotoxicity. Regulation of entry into and out of the brain plays a critical role in determining whether Mn is at a safe or toxic concentration. In serum, Mn exists as the hydrated ion, in complexes with small molecular weight ligands and bound to proteins. The spectrometry of Mn may influence the kinetics of its movement across the blood-brain barrier (BBB). This study examined the brain entry of Mn²⁺, Mn citrate and Mn transferrin (MnT), which respectively represent the hydrated, complexed and protein-bound fractions of serum Mn. The influx rates of each Mn species were determined in adult male rats using the in situ brain perfusion technique. These values were compared to predicted rates of diffusion across the BBB, which were determined from their octanol/aqueous partitioning coefficients and molecular weights. Brain influx transfer coefficients (Kᵥ values) were determined in nine brain regions and the thalamic ventricular chorioid plexus from four or five perfusion durations per species. Kᵥ values from the nine brain regions were 5-13, 3-51 and 2-13 x 10⁻¹⁰ m/s for Mn²⁺, Mn citrate and Mn T, respectively. The chorioid plexus Kᵥ values exceeded those for all brain regions by 10-fold for each Mn species. These results suggest carrier-mediated brain influx of Mn²⁺, Mn citrate and Mn T when compared to their predicted diffusion rates (1.5, 1.7 and 2.8 x 10⁻¹⁰ m/s, respectively). Kᵥ values were independent of a flow rate, determined at 10 and 20 ml/min. Mn citrate inhibited Mn citrate uptake, and Mn citrate inhibited Mn citrate uptake, and Mn citrate uptake. The greater influx transfer coefficients for Mn citrate than Mn²⁺ suggest separate influx mechanisms. These results suggest that Mn citrate uptake may be a major manganese species entering the brain. Supported by Health Effects Institute, Research Agreement P99-10.

1004 BRAIN REGIONAL INFLUX OF Fe³⁺ EFFECT OF IN VIVO MANGANESE (Mn) EXPOSURE.

R. Deane, B. Kong, J. R. Pfanner and W. Zheng, School of Chemistry & Life Sciences, University of Greenwich, London, United Kingdom and School of Public Health, Columbia University, New York, NY.

Our recent studies indicate that chronic Mn exposure is associated with an increased Fe concentration in the cerebrospinal fluid (CSF), possibly due to the action of Mn on Fe regulatory mechanisms at the blood-brain barrier (BBB) and/or the blood-CSF barrier (BCB). This study was performed to explore the relationship between Mn exposure and the influx of Fe³⁺ to various brain regions. Rat brains were infused with Ringer solution, containing traces of Fe³⁺ with or without transferrin (Tf), for 15 minutes at 37°C via the common carotid arteries. Brain regions, e.g., frontal cortex (FC), striatum (ST), hippocampus (HP), brain stem (BS), cerebellum (CB), and choroid plexus (CP) were dissected, cerebral vasculature removed by a capillary depletion method, and radioactivity assayed by a gamma counter. In control rats, addition of Tf to the perfusate greatly decreased the influx of Fe³⁺ to various brain regions. Rat brains were infused with Ringer solution, containing traces of Fe³⁺ with or without transferrin (Tf), for 15 minutes at 37°C via the common carotid arteries. Brain regions, e.g., frontal cortex (FC), striatum (ST), hippocampus (HP), brain stem (BS), cerebellum (CB), and choroid plexus (CP) were dissected, cerebral vasculature removed by a capillary depletion method, and radioactivity assayed by a gamma counter. In control rats, addition of Tf to the perfusate greatly decreased the influx of Fe³⁺ to various brain regions. Rat brains were infused with Ringer solution, containing traces of Fe³⁺ with or without transferrin (Tf), for 15 minutes at 37°C via the common carotid arteries. Brain regions, e.g., frontal cortex (FC), striatum (ST), hippocampus (HP), brain stem (BS), cerebellum (CB), and choroid plexus (CP) were dissected, cerebral vasculature removed by a capillary depletion method, and radioactivity assayed by a gamma counter. In control rats, addition of Tf to the perfusate greatly decreased the influx of Fe³⁺ to various brain regions. Rat brains were infused with Ringer solution, containing traces of Fe³⁺ with or without transferrin (Tf), for 15 minutes at 37°C via the common carotid arteries. Brain regions, e.g., frontal cortex (FC), striatum (ST), hippocampus (HP), brain stem (BS), cerebellum (CB), and choroid plexus (CP) were dissected, cerebral vasculature removed by a capillary depletion method, and radioactivity assayed by a gamma counter. In control rats, addition of Tf to the perfusate greatly decreased the influx of Fe³⁺ to various brain regions. Rat brains were infused with Ringer solution, containing traces of Fe³⁺ with or without transferrin (Tf), for 15 minutes at 37°C via the common carotid arteries. Brain regions, e.g., frontal cortex (FC), striatum (ST), hippocampus (HP), brain stem (BS), cerebellum (CB), and choroid plexus (CP) were dissected, cerebral vasculature removed by a capillary depletion method, and radioactivity assayed by a gamma counter. In control rats, addition of Tf to the perfusate greatly decreased the influx of Fe³⁺ to various brain regions. Rat brains were infused with Ringer solution, containing traces of Fe³⁺ with or without transferrin (Tf), for 15 minutes at 37°C via the common carotid arteries. Brain regions, e.g., frontal cortex (FC), striatum (ST), hippocampus (HP), brain stem (BS), cerebellum (CB), and choroid plexus (CP) were dissected, cerebral vasculature removed by a capillary depletion method, and radioactivity assayed by a gamma counter. In control rats, addition of Tf to the perfusate greatly decreased the influx of Fe³⁺ to various brain regions.
less motor activity during the hour in the figure-8 maze, relative to rats gavaged with the vehicle. The gavaged doses of the tricep metal salts did not significantly alter the performance of the rats on the step-down task. On the sequential, matching-to-sample task, rats were trained to retain a specific sequence of events over intervals ranging from 0.25 to 3 seconds. The accuracy of responding by the female rats at 30 mg/kg manganese was significantly lower at longer time intervals. In summary, the ability of manganese at 30 mg/kg to decrease accuracy of responding at longer time intervals indicates that the tricep metal at 30 mg/kg is impairing working memory on the sequential, matching-to-sample task. (Support by ATSDR grant US0/ATU398948)

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TRIGEMINAL UPTAKE AND CLEARANCE OF INHALED MANGANESE CHLORIDE IN RATS.

J.L. Lewis1, B. Tinner, G. Bercht, W. Steiner, E. Burt and K. Divine. 1Medicine, University of New Mexico, Albuquerque, NM, 2CAMS, Lawrence Livermore National Laboratory, Livermore, CA. 3Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada and 4Lovelace Respiratory Research Institute, Albuquerque, NM.

Previously we have examined uptake and transport of inhaled manganese into the brain via the olfactory epithelium. We are now investigating the role of inhalation of environmental manganese in the development of Parkinson's disease. We have expanded our investigation of the transport pathways to include analysis of the trigeminal system. Proton Induced X-ray Emission (PIXE) is used to study the uptake, deposition, and clearance of soluble manganese chloride in the olfactory and trigeminal systems following nose-only inhalation exposures. Seventy Fisher 344 rats were exposed to manganese chloride, (2.2 ± 0.5 mg/m³) for 30 min. Expositions were 6 hours/day for 10 days. Groups of rats (10 animals per group for dose 1, 6 animals per group for control) were euthanized immediately (0), 7, 14, 30, 90, 180 and 360 days post-exposure. The brain, olfactory bulbs and trigeminal tissue are removed and cryosectioned into 10 micron thick sagittal sections. To date we have completed PIXE analysis of bilateral trigeminal tissue obtained from sacrifices at 0, 7, and 14 days. Mn-dosed animals show statistically elevated levels of Mn in the trigeminal tissue at all three time points post exposure compared to vehicle controls. The maximum elevation seen in the 0 day animals decreases over successive time-points by a factor of 2, but has not reached background levels by the 14 day sacrifice. Identification of manganese within the trigeminal tissue raises the question of this pathway contributing to development of neurodegenerative diseases. This work is supported by NIHES RO1-ES10766.

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RAT STRIATAL D-ASPARTATE UPTAKE IS REDUCED BY EXPOSURE TO MANGANESE.

D. S. Barber and M. N. Morales. Physiological Sciences, University of Florida, Gainesville, FL.

Excessive exposure to manganese can cause "manganism," a neurological disorder with extrapyramidal symptoms similar to Parkinson's Disease. The mechanism underlying manganese neurotoxicity is unknown and there is no effective treatment. The targets of manganese toxicity in the nigrostriatal system raise the question of whether manganese exposure results from excessive stimulation of glutamate receptors, following manganese injection. This led us to hypothesize that manganese produces toxicity by altering glutamate neurotransmission in the basal ganglia. To test this hypothesis, male Sprague-Dawley rats were exposed to 1 or 10 mg of MnCl₂/4H₂O/ml in drinking water for 60 days. Neurotransmitter uptake was determined in crude striatal synaptosomes prepared incubated at 37°C for 2.5 minutes. Uptake of D-aspartate, a neurotransmitter in the basal ganglia, was measured at 1 or 10 mg of MnCl₂/4H₂O/ml, and was unchanged in those receiving 1 mg/ml. Dopamine and GABA uptake were not significantly altered by manganese exposure. The mechanism responsible for decreased uptake is unclear, but reduced cellular energy levels or transporter expression is likely mechanisms. The data suggest that manganese exposure causes neurotoxicity by decreasing excitatory amino acid uptake which may lead to excitotoxicity.

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PATHOLOGICAL CHANGES OF CHOROID PLEXUS FOLLOWING EXPOSURE TO LEAD AND CADMIUM IN RABBITS.

Z. E. Xu1, X. F. Li1, M. Y. Ma1 and W. Zhang1. School of Public Health, China Medical University, Shenyang, China and School of Public Health, Columbia University New York, NY.

Choroid plexus (CP) has been shown to sequester toxic metals such as lead (Pb) and cadmium (Cd). This study was performed to investigate morphological alterations in the CP following metal exposure. Rabbits received a single ip injection of 5, 25 and 50 mg Pb/kg or iv injection of 1, 2 and 4 mg Cd/kg. CFS, and blood were collected at 4 and 24 hr after dose administration for various assays and CP tissues fixed for pathological evaluation by light and electron microscopy. Concentrations of Pb and Cd were determined by AAS. Levels of glutathione (GSH) in total sulfhydryl (SH) groups were also assayed. Following exposure to Pb or Cd, the concentrations of both metals were significantly higher in the CP than those in the CFS, blood, and cerebral cortex. Under the light microscope, both metals caused vacuolation and granular degeneration in choroidal epithelia. By TEM, Pb treatment evidently altered morphology of the epithelial layer, characterized by increased numbers of lysosomes and swelling of mitochondria. Cd exposure produced abnormal choroidal convex luminal profiles, such as the loss in microvilli, increased cytoplasmic vacuolation, elevated numbers of lysosomes, dilated rough endoplasmic reticulum, and widened gaps between adjacent cells. While Pb injection increased GSH levels in the CP, Cd exposure did not affect GSH status in the CP. The concentrations of total SH groups in tissues after Pb exposure were not different from those of controls. However, the total SH level of the CP in Cd-treated group was significantly lower than that of controls. These results suggest that, if the substances of Pb or Cd in the CP can alter the morphology of the CP and that binding of toxic metals to GSH or tissue SH groups may underlie metal accumulation in this tissue. (Supported by National Natural Science Foundation of China Grant#39770642).

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INTERACTIONS BETWEEN TROPHOBLASTIC LEAD UPTAKE AND CALCIUM TRANSPORT MECHANISMS.

T. J. Evans1, M. R. James-Keager1, S. B. Keloobeker1 and S. W. Caster1. 1University of Missouri College of Veterinary Medicine, Columbia, MO and 2University of Missouri Health Sciences Center, Columbia, MO.

in utero exposure to lead (Pb) is associated with neurobehavioral abnormalities in children and laboratory animals. During pregnancy, mechanisms favoring divalent calcium (Ca²⁺) uptake by the fetus enhance mobilization of maternal Pb stores and maternal intestinal absorption of Pb. Previously, a lack of adequate Pb-specific measurement techniques has prevented real time assessment of hypothesized interactions between trophoblastic Pb²⁺ uptake and Ca²⁺ transport mechanisms. A novel method, which measures Pb²⁺-induced indo-1 fluorescence quench (I-I-FQ), was adapted for the assessment of Pb²⁺ uptake into undifferentiated and differentiated trophoblastic cells. A factorial experimental design allowed detection of significant interactions between treatments known to affect cellular uptake of Ca²⁺. Statistical analyses were performed using ANOVA, and 3 to 5 replicates of each treatment protocol were performed. Pb²⁺ uptake increased with time following the addition of higher concentrations of Pb²⁺ (10 μM versus 1 μM) and, to a much lesser degree, with lower Ca²⁺ in solution (100 μM versus 1 μM). Activation of capacitative Ca²⁺ entry by 10 μM thapsigargin enhanced Pb²⁺ uptake into Hro-1 cells, and this mechanism of Pb²⁺ influx was inhibited by 10 μM La³⁺. Incubation with the midportion of parathyroid hormone-related peptide (PTPH) in an endocrine stimulus for Ca²⁺ uptake by Hro-1 cells, increased the uptake of Pb²⁺ into undifferentiated Hro-1 cells. The lower relative rate of I-I-FQ in differentiated compared to undifferentiated, Hro-1 cells was unexpected. This observation could be explained by binding of PTHP to Ca²⁺-receptor-associated Ca²⁺-binding proteins instead of I-I-FQ. Uptake of Pb²⁺ by Hro-1 cells was not affected by interactions between differentiation status and other treatments known to impact cellular uptake of Ca²⁺. The hypothesized facilitation of trophoblastic Pb²⁺ uptake by Ca²⁺ transport mechanisms was supported by these results.

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BLOCKAGE OF COPPER RELEASE BY LEAD IN ASTROGLIA VIA TARGETING HEAVY METAL BINDING DOMAIN OF ATP7A.

Y. Qian, Y. Zheng and E. Tiffany-Castiglione. Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

Lead (Pb) is well known developmental neurotoxicant that may also be a risk factor for neurodegenerative disorders. Astroglia are the major site in brain for Pb accumulation. Astroglia are also the principal loci for other metals, such as copper (Cu) in Menke's disease, which offers a link for understanding some of the neurotoxic mechanisms of Pb. The central hypothesis of our work is that intracellular Pb exerts multiple neurotoxic effects by interfering with Cu transport and buffering mechanisms in astroglia. This hypothesis is based on compelling previous evidence from our laboratory that Cu accumulation in brain and in astroglial cultures is associated with Pb exposure, and that Cu efflux from C6 cells may be blocked by the binding of Pb to ATP7A (Menke's protein), a Cu/ATPase. We partially purified the heavy metal binding region (HMB) of ATP7A that was overexpressed in E. coli, and showed that Pb has a stronger binding affinity than Cu for this construct. One molecule of HMB was found to chelate 11 Pb ions or 7 Cu ions, and Pb competitively bound HMB in the presence of Cu. These findings support the conclusion that Pb increases intracellular Cu levels in differentiated astroglia by blocking ex-
LEAD-INDUCED ALTERATIONS OF DOPAMINERGIC SIGNALING SYSTEMS IN PHEOCHROMOCYTOMA CELLS.

L. M. Gardiner and A. L. Jadav. College of Pharmacy, Texas Southern University, Houston, TX.

Lead-induced alterations of the dopaminergic neurotransmitter system in specific brain regions appear to be closely associated with manifestations of behavioral changes. Moreover, alterations of the rate-limiting enzyme of the dopaminergic biosynthetic pathway, tyrosine hydroxylase (TH), have been associated with low-level lead exposure in various animal and cell models. However, the underlying biochemical and molecular events leading to these changes are obscure. Therefore, the objective of this study was to determine the effect of lead on the dopaminergic signaling system (i.e. Calcium/Calmodulin dependent TH phosphorylation and activity) in pheochromocytoma (PC12) cells. PC12 cells are a particularly useful in vitro tool for this type of study because they synchronize, store, and secrete appreciable amounts of dopamine. The results of this study revealed dose-dependent decreases of dopamine levels at 0.1, 0.5, 1, 5, and 10 μM lead exposure, followed by a dose-dependent increase to near control level at 10 μM lead exposure. CaMK II β protein expression exhibited significant increases from the control level at 0.5 and 5 μM of lead exposure, followed by significant decreases at 1 and 10 μM of lead exposure. There were overall decreases of tyrosine hydroxylase phosphorylation, and tyrosine hydroxylase activity with significant changes occurring at 5, 1, 0.5, and 1 μM lead exposure, respectively. CaMK II activity exhibited biphasic changes with a significant increase at 0.5 and significant decreases at 5 and 1 μM lead exposure further, this study revealed that 1 μM lead decreased CaMK II β expression and inhibited CaMK II activity. The inhibition of CaMK II activity was associated with attenuated TH phosphorylation and TH activity, which subsequently led to decreased dopamine levels. Hence, these findings suggest that lead-induced decreases in dopamine levels in PC12 cells may be due to its effects on the CaMK II decrease in dopamine levels and TH phosphorylation and TH activity. Supported by ATSDR, Cooperative Agreement #U50/ATU 3080948 and NIEHS, Cooperative Agreement #ES07290.

ASSESSING THE ROLE OF THE DORSAL SUBICULAR/NUCLEUS ACCUMBENS PATHWAY IN FB-INDUCED CHANGES IN FIXED INTERVAL (FI) PERFORMANCE.

M. B. Virgolini and D. A. Cory-Slechta. Environmental Medicine, University of Rochester, Rochester, NY.

Previous studies from this laboratory have demonstrated that Pb-induced decreases in FI response rates are related to dopaminergic/glutamatergic alterations in nucleus accumbens (NAc). NAc integrates dopaminergic inputs from the lateral septal area with glutamatergic inputs from the hippocampus (Hipp) and prefrontal cortex (PFC), areas considered critical in the regulation of cognitive and motivational behaviors. An anatomical disconnection procedure was used to better delineate the circuits involved in regulating FI performance. Rats trained on a FI-1 minute schedule of food reward were bilaterally implanted with guide cannulae in NAc core and the dorsal subiculum (DSb) area of Hipp. Lidocaine was microinjected unilaterally in NAc core and on the contralateral side of DSb (disconnection), temporarily inactivating the circuit, while leaving operant glutamatergic circuits connecting VShb with PFC and PFC with NAc. Lidocaine disconnection decreased FI response rates, but did not affect post-reinforcement pause time. To test whether preferential AMPAergic activation of the operant DSb to PFC to NAc circuit could reproduce Pb-induced decreases in FI response rates, doses of MK 801 (1.0-5.0 mg) or AMPA (0.03-0.3 mg) were injected in the non-lidocaine side of DSb in conjunction with the disconnection. This procedure likewise lowered rather than increased FI response rates. Collectively, these findings suggest that DSb/NAc pathway could play a role in increased FI rates following disconnection of this circuit. But preferential AMPA receptor activation of the DSb to PFC to NAc pathway may not be the operative mechanism, since it did not reproduce increased FI rates. Supported by ES01247, ES05017 and ES05903.

LEAD-INDUCED AGGRESSIVE BEHAVIOR IN ADULT MICE.

L. Y. Calvin, D. Hoover and E. K. Silbergaedl. Toxicology, University of Maryland at Baltimore, Baltimore, MD.

Lead is a heavy metal hazardous to both children and adults. Lead has no biological benefit to the body and exposure to the body can produce a range of toxic effects, especially in the nervous system. While some epidemiological studies have associated lead exposure with aggressive behavior, there has been little research in animal models. Experimental research on aggression in rodents has utilized mostly qualitative descriptions of the behavior. No method for quantitatively measuring the amount of aggressive behavior has been reported. We have developed a method for quantitative assessment of aggression in mice, and we report on the effects of lead exposure on aggressive behavior. Adult male and male CD-1 mice were treated with 0.25% or 0.5% lead acetate for 3 or 5 weeks. They were paired by gender for behavioral testing with or without isolation. Behaviors were videotaped for 5 minutes and evaluated using a scoring system. The system was validated in studies of isolation-induced behavior and studies of baldwinius as an anti-aggressive agent. Our results revealed quantitative differences in aggressive behavior between male and female mice exposed to lead suggesting involvement of the hormonal system. These results permit future investigations on the mechanism(s) underlying lead-induced aggressive behavior, which may be an important public health impact of lead exposure. Supported by A Ronald McFarland Fellowship to Lynn Calvin as an undergraduate student at Coppin State College.

METHYLMERCUry IMPAIRS NEURONAL DIFFERENTIATION BY ALTERING NEUROTYPHIN SIGNALING.


In previous in vitro studies, we observed that developmental exposure to CH₃Hg can alter neurotrophic morphology and neurotrophin signaling. Using primary PC12 cells as a model system for neuronal differentiation, we examined the hypothesis that the developmental effects of CH₃Hg may result from inhibition of neurotrophin signaling through the Trk receptors. Stimulation of PC12 cells with nerve growth factor (NGF) for 24 h resulted in robust neurite outgrowth, which was inhibited by CH₃Hg in a concentration-dependent manner (EC50 = 0.03 μM). Whole cell binding assays using [125I]-NGF revealed a single binding site with KD of ~1 μM. Exposure of PC12 cells to CH₃Hg (0.001 - 3 μM) had no effect on NGF binding. Following NGF binding, TrkA receptor autophosphorylation peaked at 2.5 min of NGF stimulation and was sustained up to 60 min. Concurrent exposure to CH₃Hg for 2.5 min resulted in a concentration-dependent decrease in TrkA autophosphorylation, which was significant at 0.1 μM CH₃Hg (50%). To determine whether inhibition of TrkA activation affected downstream signaling, activation of MAP kinase (ERK1/2) and Protein Kinase C (PKC) were examined. Similar to TrkA, NGF-stimulation resulted in a time-dependent activation of TrkA autophosphorylation, which was significant at 0.1 and 0.3 μM (~75%). While CH₃Hg inhibited PKC activity for several recombinant PKC isoforms (δ, ε, and γ) and in PC12 cell homogenates, there was no effect on NGF-stimulated PKC activity in whole cells. To correlate the neurochemical and morphological effects, PC12 cells were exposed to specific inhibitors of TrkA (K252a) and ERK1/2 (U0126) at concentrations selected to produce 50-75% inhibition. Both K252a and U0126 significantly inhibited components of neurite outgrowth. These data suggest that the inhibition of neurite outgrowth by CH₃Hg may be related to a disruption of the NGF signaling through the TrkA receptor.

USE OF P53 GENETICALLY ENGINEERED PC12 CELLS FOR TESTING CHRONIC METHYLMERCURY NEUROTOXICITY.

C. Eskele, J. Gardlon, S. Singele, D. Sladowski and S. Cooceke. ECVAM, Institute for Health & Consumer Protection, European Joint Research Center, Ispra (VA), Italy and Department of Transplantology, Warsaw University School of Medicine, Warsaw, Poland. Sponsor: L. Schelling.

Methymercury is an environmental hazard substance known to induce neurotoxic effects. Apoptosis is known to be one of the mechanisms by which methymercury induces neural cell death. The expression of the p53 gene is often described to be implicated in the induction of apoptotic mediated cell death. A novel genetically engineered PC12 cell line was developed at ECVAM (Singele et al., 1999), which has a controlled expression of the p53 gene according to the media used. The present work was undertaken to test whether p53 expression is implicate in chronic methymercury toxicity. After application of NGE19 induce cell differentiation and acquisition of neuron-like characteristics, cells were subchronically treated for 10 days with 10⁻⁶ M to 10⁻¹ M of methymercury. Results were compared to previous experiments carried out on primary brain cell cultures. The use of this novel genetically engineered PC12 cell line in an integrated testing strategy for evaluating chronic neurotoxicity of xenobiotics is discussed. Singele S, Cooceke S, Nicotera P & Balls M. (1999) EC Patent filed 27th of
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STIMULATION OF ARACHIDONIC ACID RELEASE BY METHYLMERCURY IN PRIMARY CULTURES OF ASTROCYTES AND NEURONS.

G. Shanker, I. A. Mudros, K. H. Tan and M. Aschner. Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC.

Cytosolic phospholipase A2 (cPLA2) stimulates the production of arachidonic acid (AA) from the sn-2 position of membrane phospholipids, the former serving as a precursor for the synthesis of prostaglandins. cPLA2 is upregulated in ischemia, epilepsy, and Alzheimer’s disease, as well as by the excitotoxic neurotransmitter, glutamate. Glutamate upregulates transporter inhibition and a known consequence of Aβ-mediated reactive oxygen species generation. The enzyme cPLA2 has been a target of therapeutic strategies examining the effect of methylmercury (MeHg) on astrocytic cPLA2 activation and AA release, or on the overall significance of this pathway to MeHg-induced neurotoxicity. In the present study, primary cultures of astrocytes and neurons were exposed to 30 μM MeHgCl for 3 hours. The enzyme cPLA2 was evaluated by measuring the release of arachidonic acid (AA) from astrocytes or neurons over 24 hours.

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MULTIPLE FACTORS CONTRIBUTE TO THE METHYLMERCURY (MEHG)-INDUCED EARLY STIMULATORY EFFECTS ON SPONTANEOUS SYNAPTIC CURRENTS OF PURKINJE CELLS IN CEREBELLAR SLICES OF RAT.

Y. Yuan and W. D. Attickson. Pharmacology/Toxicology, Michigan State University, East Lansing, MI.

Both application of 20-100 μM methylmercury (MeHg) initially stimulates and then suppresses the frequency of spontaneous excitatory and inhibitory synaptic currents recorded from Purkinje cells in rat cerebellar slices using whole cell patch clamp recording techniques. To determine whether the early stimulatory effect is the result of MeHg-induced elevation of intracellular Ca2+ concentration ([Ca2+]i), which increases release of neurotransmitter, the effect of MeHg on the frequency of spontaneous synaptically evoked currents was examined in reduced extracellular Ca2+ concentration (2 μM). Reduction of [Ca2+]i from 2 to 0.2 μM did not significantly affect MeHg-induced early increase in the frequency of spontaneous synaptic currents, suggesting that Ca2+ influx is not a major contributor to the MeHg-induced early stimulatory effects. It is possible that increased release of Ca2+ from the intracellular stores is involved. To test this, ruthenium red (RR), an inhibitor of mitochondrial Ca2+ transport, was used to prevent the MeHg-induced release of transmitter. Preincubation of cerebellar slices with 20 μM RR diminished, but did not prevent MeHg-induced stimulatory effects on spontaneous synaptic responses. Preincubation of cerebellar slices with the membrane-permeable Ca2+ chelator BAPTA AM (40 μM) also failed to prevent MeHg-induced increases in spontaneous synaptic responses, suggesting that increased [Ca2+]i is not the only factor contributing to the early stimulatory effects. Treatment of cerebellar slices with 40 μM TPN, which chelates zinc but not MeHg, significantly reduced MeHg-induced increases in the frequency of spontaneous currents with larger amplitudes, but not those with smaller amplitudes, suggesting that the intracellular zinc may also play a role in MeHg-induced early stimulatory effects. Thus, multiple factors may be involved in the early stimulatory effects of MeHg on spontaneous synaptic currents in cerebellar Purkinje cells. Supported by NIH grant ES03299.

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METHYLMERCURY-INDUCED BLOCK OF VOLTAGE-GATED Ca2+ CHANNELS IN ACUTELY ISOLATED CEREBELLAR PURKINJE NEURONS OF RAT.

S. Q. Peng, A. Z. Yao and W. D. Atkinson. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

Cerebellar granule cells are more susceptible to the toxicity of methylmercury (MeHg) than are Purkinje cells. Voltage-gated Ca2+ channels are a prominent target of MeHg. To determine if the presence of a unique type of Ca2+ channel, or a differential expression of Ca2+ channels, confers upon granule cells and Purkinje cells a differential sensitivity to MeHg, we examined the effects of MeHg on current of voltage-gated Ca2+ channels in acutely dissociated Purkinje neurons from 7-11 day-old rats, using whole-cell recording techniques. Ba2+ currents in Purkinje cells were blocked 18.9±2.4%, 27.0±3.6% and 56.4±1.7% by nifedipine (1.0 μM), by a-cytoxin-GVIA (1.0 μM) and a-gatoxinIVA (50 nM) after 3 min exposure, respectively. Exposure of Purkinje cells to MeHg (0.5-5 μM) reduced peak and end currents in both time and concentration-dependent manner. At 0.5 μM, the effect of MeHg was greater on the sustained current at 100 ms time point than on the peak current. MeHg accelerated the time course of inactivation of the current. At 1.0 and 5.0 μM, the degree of block by MeHg was approximately the same for both peak and sustained components of current. Whole cell currents were reduced in amplitude by 95% after 3 min exposure to 5.0 μM MeHg. The current-voltage relationship was altered after 5 min exposure to MeHg. However, the steady-state inactivation relationship was shifted to more negative potentials without a shift with MeHg-free solution did not reverse effects of MeHg. Increasing the stimulation frequency from 0.1 Hz to 0.2 Hz did not accelerate the block of I_B by MeHg. Comparing these results with previous results from granule cells (Sirois and Atkinson, Toxicol Appl Pharmacol. 109:1-11, 2000), the sensitivity of Purkinje cell Ca2+ channels to MeHg was about the same as that in granule cell. Thus, it is unlikely that the presence of a unique Ca2+ channel subtype or differential complement of Ca2+ channels renders granule cells more susceptible to neurotoxicity of MeHg. Supported by NIH grant ES03299.

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EFFECTS OF METHYLMERCURY EXPOSURE ON EXPRESSION OF EPHS AND EPHRINS IN EMBRYONAL CARCINOMA CELLS.

D. T. Wilson, K. K. Reuhl and R. Zhou. JIGF and Neurotaxis Lab and Department Chem. Biol., Rutgers University, Piscataway, NJ.

Repulsive interactions mediated by Eph receptors and ephrin ligands guide many morphogenetic processes, including the establishment of proper topographical mapping of various pathways within the CNS. Methylmercury (MeHg), a potent environmental neurotoxicant, induces morphological changes in the developing brain indicative of path-finding errors. The effects of MeHg on EPHs and ephrins were examined in P19 embryonal carcinoma (EC) cells and in neurons derived from P19 cells by retinoic acid induction. Undifferentiated EC cells and day 5 neurons were exposed to 0.5 μM MeHg in the cell culture media. Following 24-hour exposure, total RNA was isolated from undifferentiated P19s or day-6 neurons and RNase protection assays were performed. Levels of EPH and ephrin mRNA were evaluated using the Ribonuclease protection assay system with receptor and ligand probe template sets. Undifferentiated EC cells expressed only ephrins-B2 and A5 and this expression was abolished by MeHg treatment. MeHg exposure also decreased expression of ephrins-B2 and A5 in day 6 neurons compared to control, while expression of ephrins B3, B1, A6, A4, A3, and A1 remained unchanged. EPHs A1 and B3 were the only receptors detected in undifferentiated cells. EPHA1 expression was enhanced in MeHg treated cells while EPHB3 expression was decreased. EPHA1, A2, A3 and A4 expression was decreased in MeHg treated day 6 neurons while remaining receptors appeared to be unaffected. These data suggest that selective perturbation of repulsive guidance molecules may play a role in the brain morphogenesis caused by MeHg. (Supported by ES05022 and T7148).

1022

THE EFFECT OF GESTATIONAL MERCURY VAPOR EXPOSURE ON RAT BRAIN α-SYNUCLEIN EXPRESSION.


α-Synuclein is a highly conserved protein that localizes to pre-synaptic terminals and is thought to play a role in neuronal plasticity. It is upregulated developmentally and continues to be expressed at high levels in the adult brain. Its presence in a number of neuronal (AD, PD, dementia with Lewy bodies) and glial (Multiple System Atrophy) pathologies has led to the naming of a class of specific neurodegenerative diseases known as synucleinopathies. Alpha-synuclein has been implicated in a number of possible neurodegenerative mechanisms ranging from oxidative stress to changes in neurotransmitter processing. In addition, the pesticides rotenone, dieldrin and paraquat have been reported to increase the rate of synuclein fibril formation, similar to those found in amyloid plaques of AD and Lewy bodies of PD. These factors make cerebellum a likely participant in environmentally induced neurotoxicity. It is well accepted that exposure to methyl mercury chloride has critical consequences in the developing brain. Recently concern has been raised that exposure to mercury vapor may be similarly neurotoxic (Fredrickson et al., 1996). Pathological changes such as decreases in cortical layer widths, diminished cell size and number and the induction of gliosis (Barone et al.,)
1998) as well as behavioral changes in somatosensory information processing have been reported. A study was designed to examine the consequences of gestational (GD 6-15) mercury vapor exposure (4 µg/m3) on neuroanatomical and neurological endpoints. Here we report on the consequences on α-synuclein protein expression and 2 years post-natal in cortical, striatal and cerebellar regions of the rat brain. Data do not support long-term changes in brain protein levels of α-synuclein after gestational mercury vapor exposure. However, this does not eliminate the possibility of early perturbations in the formation of synaptic connections that might result in long-term pathology. This abstract does not necessarily reflect USEPA policy.

1023 BEHAVIORAL EFFECTS OF EXPOSURE TO Hg³⁺ FROM DENTAL AMALGAM.

D. Echeverria¹, N. Fleyer², A.C. Bittner², L.S. Wood⁴, D. Rohlman⁵ and K. Angert⁶. "Centers for Public Health Evaluation, Bastelle, Seattle, WA" and "CREOT, Oregon Health Sciences, Portland, OR".

Potential neurotoxicity from exposure to mercury vapor (Hg⁴⁺) from dental amalgam fillings is a subject of public health debate. A urinary Hg (HgU) screen of 4, 750 dentists in Washington State yielded 2, 835 samples with a range of 0-59 and mean (sd) of 2.51 (3.01) µg/l HgU. Among 1, 488 eligible dentists, 200 dentists and 200 assistants were enrolled to be representative of Hg exposures in the population (DDE = 3.08 (2.00); DA = 1.96 (1.82) µg/l HgU). A repeated cross-sectional behavioral study was conducted six months apart at a study center. Sessions included an evaluation of symptoms (transient and persistent), mood, cognitive, motor, and peripheral nervous system function in relation to HgU, applying the Behavioral Evaluation for Epidemiology Studies (BEES) touch-screen test battery. Hold tests included Vocabular, WRAT, and visual acuity. Nitrous oxide (mean ppm = 1.94, (1, 611) was measured in air. Multiple regression models were analyzed. A significant (p<0.05) effect was most pronounced for mood (Feek Depression Index and the SCL90 (Beta = 0.38 and .20 respectively) and symptoms (Beta = 0.18), followed by motor function (Hand Steadiness (Beta = 0.19) and Finger Tapping (Beta = 0.18). Selective cognitive effects were found for Tracking and Trails A (both Beta = 0.19), Visual Reactions and Symbol-digit (both Beta = 0.15), and Digit Span (Beta = 0.11). Visual Contrast, Smell, and Nerve Conduction Velocity (1/µs) also achieved significance (all Beta = 0.14). No effects were found for Trails B, Pattern Recognition and Memory, Reaction Time, Vigilance, and Vibration Sensitivity. Models controlled for age, alcohol, medications, and glasses. Mean dental urinary Hg levels for the USA (3.50 µg/l) Washington State (3.01 µg/l), and the general population (-3.39 µg/l) are consistent with levels at which we have first observed significant CNS deficits (1.5 µg/l) justifying characterization of chronic exposure to determine a safe level.

1024 MITOCHONDRIAL MEDIATED APOTOPSIS IN A HUMAN NEUROBLASTOMA CELL LINE (SK-N-SH) BY THIMEROSAL: A PROAPOPTOTIC ROLE FOR NUCLEAR FACTOR KAPPA BINDING PROTEIN (NF KB).

K. K. Kinningham¹, M. P. Cole¹, J. C. Pendergass² and D. K. Si, Chitt³. "Graduate Center for Toxicology, University of Kentucky, Lexington, KY" and "Affinity Labeling Technologies, Inc., Lexington, KY".

The objective of this study was to examine the pathway of cell death in SK-N-SH cells by thimerosal, an ethylmercury containing preservative used in pharmaceutical preparations such as vaccines. Inorganic and organic mercury compounds cause apoptosis in cultured neurons; however, the signaling pathways resulting in programmed cell death have not been reported. SK-N-SH cells were exposed to thimerosal (0-10 µM) for 24 hours and then assayed by DNA fragmentation analysis. Cells treated with 3-10 µM thimerosal exhibited 250-300 bp nucleosomal fragments. To determine if the death pathway was mitochondrial mediated, JC-1 fluorescence, cytochrome c, and AIF were analyzed. A significant decrease in JC-1 fluorescence was noted within 2 hours of treatment. Cytochrome c and AIF were shown to leak from the mitochondria in a dose dependent manner. Caspase 3 cleavage occurred within 4 hours of treatment followed by caspase 3 activation. Within 12 hours poly(ADP)ribose polymerase, a downstream target of caspase 3 was cleaved to form 85 and 64 kDa proteolytic fragments. These results suggest a mitochondrial mediated apoptotic pathway in SK-N-SH cells upon thimerosal exposure. Reactive oxygen species (ROS) which contribute to mitochondrial mediated apoptosis increase upon mercurial exposure. A dose dependent increase in 4-hydroxy-2-nonalanlydral adducted proteins was observed, indicative of ROS formation and lipid peroxidation. Sustained levels of the redox sensitive transcription factor, NF KB, was observed with 10 µM thimerosal. However, treatment suppressed superoxide dismutase, a NF KB target gene and antioxidant known to attenuate mitochondrial mediated apoptosis, was not induced suggesting a proapotptic role for the transcription factor. In summary, this study identifies NF KB as a potential pharmacological target for intervention of thimerosal mediated neurotoxicity.

1025 THIMEROSAL INDUCES MICROTUBULE DEPOLYMERIZATION IN DEVELOPING NEURONS.

R. N. Grisham¹, S. S. Shah¹ and K. R. Reuhl. "Pharm. and Toxicol., Rutgers University, Piscataway, NJ".

Thimerosal has been widely used in vaccines as an antimicrobial and preservative. Recently, its use has come under scrutiny due to the potential neurotoxicity of its chief component, ethylmercury, but its exact mechanism of toxicity is unknown. Thimerosal acts as a sulfhydryl binding agent and increases intracellular calcium levels. Since the microtubule (MT) network is highly susceptible to perturbations by SH agents and/or elevations of cytosolic calcium, disruption of MT-dependent functions may underlie the compound's toxicity. The effects of thimerosal and a known anti-MT agent, methylmercury (MeHg) on MTs of undifferentiated cells and neurons were compared using P19 embryonal carcinoma cells. EC cells differentiate into neurons following induction by retinoic acid (RA). EC cells and developing neurons were exposed to 0.4-5 µM thimerosal or MeHg for 2-24 hrs, then fixed or allowed 22 hr recovery in control medium prior to staining with FITC-conjugated anti-tubulin (clone DM1A) or neuron-specific βIII tubulin. MTs were examined by epi-fluorescence microscopy. Thimerosal induced robust disassembly of MTs in both undifferentiated cells and developing neurons. In EC cells, loss of MTs occurred at thimerosal concentrations as low as 0.1 µM and MTs were completely lost at 1.5 µM and above. In developing neurons, 1.5 µM disassembled most perinuclear MTs and induced retraction of neuritic processes. At 2.5 µM and above, MTs were completely absent and no neurites were observed. Both undifferentiated cells and developing neurons were treated for 24 hrs with MeHg induced MT disassembly, with complete loss of MTs occurring at 2.5 and 3.5 µM, respectively. Recovery of MTs in thimerosal-treated cells incomplete even after 22 hrs and levels above 2.5 µM appeared lethal to most cells. In contrast, substantial MT recovery was observed in MeHg cells up to 2.5 µM. Data indicate that thimerosal is more toxic than MeHg to MTs in undifferentiated and neuronal cells. Effects on MTs may play a significant role in the toxicity of thimerosal. (Supported by NJ Governor's Council on Autism and ES 05022)

1026 RESPONSE OF EPENDYMAL STEM CELLS TO NEUROTOXIC DAMAGE.

B. C. Weig¹, M. W. McBurney², H. E. Lowndes¹ and K. R. Reuhl. "Pharmacology and Toxicology, Rutgers, The State University of New Jersey, Piscataway, NJ" and "Ontario Regional Cancer Center, Ottawa, ON, Canada".

Numerous studies have confirmed the presence of neural stem cells capable of forming neurons within the adult brain. These cells have been isolated from several cortical regions, including hippocampus and the ependymal/subventricular zones. To test the hypothesis that neurotoxic injury may signal proliferation and migration of ependymal stem cell progeny as part of a repair response in the brain, C3H mice were unilaterally injected intracerebrally with the lipophilic fluorescein dye DiI and given the hippocampal neurotoxicant trimethyltin (TMT). Brains were removed 2 days post-injury and BrdU was administered daily (tp) to label proliferating cells. Brain sections from mice sacrificed 3, 7, 14 and 21 days post-TMT were examined by fluorescence microscopy. DiI labeling appeared confined to ependymal cells and their progeny. At 7 days post-injury, DiI-labeled cells were observed migrating from the subventricular zone toward the hippocampus and by day 14 Di-labeled cells were observed within the inner boundary of the dentate gyrus. The ependymal lining adjacent to migrating DiI-labeled cells often exhibited "pitting" and decreased fluorescence implicating the ependymal layer as their point of origin. NeuN+/BrdU- cells were visible in the dentate gyrus and subependymal zone 3 days after TMT treatment; however, at this time these cell were DiI-. A marked astroglial response consisting of GFAP+/BrdU- cells was seen in the hippocampus throughout the study. These results suggest two phases of neuronal replenishment occur following neurotoxic injury. The rapid first phase involves cells resident within the dentate while the second, slower phase involves activation and recruitment of cells originating in the ependymal layer. The relative contribution of the two phases to the final repair of damaged neural tissues remains to be determined. (ES01513, ES07148, ES05022)

1027 INHIBITION OF NEURONAL OUTGROWTH IN HIPPOCAMPAL NEURONS IN CULTURE BY TRIMETHYLtin.

H. A. Garcia¹, S. S. Lundback², S. R. Heidemann³ and W. D. Archigan¹. "Department of Pharmacology/Toxicology, Michigan State University, East Lansing, MI" and "Department of Physiology, Michigan State University, East Lansing, MI".

Trimethyltin (TMT) is an organomineral neurotoxicant, which specifically affects pyramidal neurons in the hippocampus. The exact mechanism of toxicity of TMT is as yet unknown. The objective of this project was to examine the effect of TMT on
neural outgrowth, a developmental process, in hippocampal pyramidal cells in primary culture. Hippocampal neurons go through a highly stereotyped developmental sequence leading to a predictable outgrowth of axons. Stage I involves generalized motility. Stage 2 involves the outgrowth of short minor processes and Stage 3 involves the outgrowth of a long axon. Hippocampal cells isolated from 18 day old rat embryos were exposed in vitro for 24 hrs to 0.125µM, 0.25µM, 0.5µM, 1µM, or 2µM TMT, or TMT-free solutions as a control. TMT was subsequently removed, and photos of developing cells were taken at 24, 48 and 72 hrs after plating to assess the number of cells in each stage counted. TMT-free solutions did not promote the outgrowth of hippocampal neurons at 24, 48 or 72 hrs. By 72 hrs, ~65% of the cells had progressed to Stage 3, and less than 20% remained in Stage 1. In contrast, TMT had a concentration-dependent action to suppress neural processes. Outgrowth of hippocampal neurons. At low concentrations (0.125-0.5 µM) TMT interfered with the progression of neurons from Stage 1 to subsequent stages. By 72 hrs ~25-35% of the cells had progressed to Stage 3. However, at 1 and 2µM TMT, the slowing of growth progression was more prominent, and more prolonged. Less than 25% of the cells progressed to Stage 3 under these conditions, and 40-50% of the cells remaining in Stage 1. Thus, acute exposure to TMT can affect the processes of neuronal growth and differentiation in hippocampal neurons in culture. Supported by NIH grant R03-ES10716.

1028
AUTOANTIBODIES TO NEURONAL SYSTEM PROTEINS FOLLOWING TRIMETHYL TIN (TMT) EXPOSURE: A COMPARISON OF ELISA AND WESTERN-BLOT ANALYSIS.


1 Pharmacology and Toxicology Laboratory, Mercy College, Dobbs Ferry, NY; 2Environmental Medicine, NYU Medical Center, Tuusalo, NY and 3Molecular Neurotoxicology Laboratory, CDC-NIOSH, Morgantown, WV.

Previous studies in both human and animals have demonstrated the presence of serum autoantibodies to neurotropic (e.g., neurofilament triplet (NFL)) and glial proteins (myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP)). Following exposure to some heavy metals, solvents, or pesticides, TMT has been used as a dervantive tool to validate the enhanced expression of GFAP as a biomarker of astroglial resulting from neuronal damage and cell death. In particular, TMT targets hippocampal pyramidal neurons in CA3 and CA1. In the present study TMT was used to assess the detection of the serum polyclonal IgG responses against NFTs, measured by ELISA, as a peripheral marker of neurotoxicity. Western-blotting, using purified NF proteins, or hippocampal homogenates, was used to confirm ELISA. Male Long-Evans rats (45 days of age) were administered either TMT (8mg/kg; n=10) or an equal volume of sterile 0.9% saline (n=10). At 3 weeks post-administration, serum was collected and rats were sacrificed for the collection of brains. The polyclonal IgG response to NF65, NF160, and NF200, using ELISA, showed detectable titers of IgG autoantibodies to NFs in sera from TMT-exposed rats, only. Ancl-NF60 titers were highest compared to NF160, or NF200. Immunostaining of Western-blots using HRP-conjugated anti-rat IgG confirmed the presence of antibodies, against purified proteins or hippocampal homogenates, in sera of rats exposed to TMT. This corresponded to the molecular weights of NF68, NF160, and NF200. Immunohistochemistry of the TMT-exposed brain showed that the antibodies recognized a specific region of the hippocampus, a means of detecting the autoantibody response. This study suggests that the detection of autoantibodies to neurotropic proteins, using ELISA, may be used to indicate chemical neurotoxicity. (Supported by NIH HD36355 and USDF P217A909192).

1029
ZINC-INDUCED MODULATION OF CHOLINE TRANSPORT IN CULTURED CHOROID PLEXUS.


Removal of choline from cerebrospinal fluid (CSF) by choroid plexus is a potentially critical regulatory point in central cholinergic homeostasis. Modulation of the transport pathway by chemical stress may alter choline availability in the CNS. We examined the potential effects of zinc on choline removal from CSF in primary cultures of neonatal rat choroid plexus epithelium. Cultures were exposed to 10 µM ZnCl2 in serum-free medium for 1.5 hrs (37°C). After 0.12 hrs exposure in Zn-free medium/5% N2 urban a severe heat shock (SHS, 45°C, 1 hr) ventricular uptake of 10 µM 3H-choline a 750 µM hemicellulose-3 was assayed in artificial CSF (30 min, 37°C). LDL release was assayed to evaluate cytotoxicity. HPS70 accumulation was analyzed by immunoblot and normalized to total RNA. To assess cytoskeleton integrity and general cell morphology, actin was probed with TRITC-labeled phalloidin and viewed by fluorescence microscopy. After Zn exposure choline uptake was comparable to that by untreated controls with LDL release increasing 12%. However, uptake was stimulated 15% after 1-h recovery. Stimulation persisted through 12-h recovery, at which time uptake was enhanced 50%. Thermoresistance of transport was also enhanced with recovery. Without Zn recovering, SHS increased LDL release <5% but reduced choline uptake 50%. However, after Zn exposure, SHS reduced uptake by only 25%. In cultures subjected to SHS after 1-h recovery, uptake was comparable to that by non-SHS controls. After 12-h recovery, SHS reduced uptake by 20%. Thus, complete chemo-protection was transient. Zn exposure also induced HPS70 accumulation, with levels peaking 3-6 h post Zn exposure and declining thereafter. While overall cell morphology was maintained, Zn-exposure induced migration and localization of actin filaments to the cell periphery. These data suggest that zinc may induce latent signaling pathways of cholelon plexus release of choline from CSF, possibly correlated with induction of HPS70 and the actin cytoskeletal reorganization. ES10439; NS39452.

1030
TRANSPORT OF ZINC IN THE OLFACTORY PATHWAYS OF RATS.


The olfactory route provides a pathway for metals to the brain, thus allowing them to circumvent the blood-brain barrier. In the present study zinc-65 was instilled intranasally in rats and the disposition of the metal in the olfactory system was examined. Autoradiography with tape-sections showed a transport of the metal to the terminals of the primary olfactory neurons in the glomeruli of the olfactory bulbs, followed by a passage across the synapses to the interior of the bulbs. Cell fractions showed that the zinc in the olfactory epithelium and the olfactory bulbs was present both in the cytosol and in association with various particulate cell constituents. Gel filtrations of the cytosolic fractions on a Sephadex 30 column showed that the zinc was eluted in the void volume (MW >16,000 Da). Intranasal pre-treatment of rats with cadmium, to induce metallothionein, did not affect the subcellular distribution of zinc. Our results show that zinc is transported along the olfactory pathway. The data indicate that during this process the metal is bound to both particulate cellular constituents and cytosolic components, the latter having a high molecular weight.

1031
ENVIRONMENTAL RISK FACTORS FOR ALZHEIMER'S DISEASE.

K. J. Ghosal, O. K. Siddiqi, and N. H. Zawia. Biomedical Sciences, URI, Kingston, RI.

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder, characterized, in part, by the extra-neuronal accumulation of beta-amyloid peptide. This peptide is a cleavage product of the amyloid precursor protein (APP). Aggregation of this peptide is suspected to lead to neuronal damage and cell loss. The factors that result in the aggregation of this peptide are not known, however, it is possible that exposure to environmental agents could promote such aggregation. Previous studies have alluded to the presence of zinc and potentially other metals in the amyloid plaques (Bush et al., JBC, 2000). In an attempt to identify environmental contribut- ing factors to this process, we screened a variety of metals and tested their ability to enhance beta-amyloid aggregation. Synthetic Abeta 1-40 peptide was obtained and used in an assay to determine its aggregation in the presence of a series of metals. The assay involved measuring Abeta aggregation by examining the fluorescence spectra that are produced at 482 nm only when the aggregated peptide binds to the Thilinlan 1 probe. Initially the aggregation of the peptide was studied by varying the pH (pH 3-8) of the buffer. Subsequently, various metals were tested for their ability to enhance aggregation beyond that produced by pH alone. The metals studied were Zn, Pb, Mn, Ba, Tb and Mn promoted aggregation at pH 5-6, at a concentration of 10µM, while both Zn and Ba had minimal effects at this concentration. These preliminary results suggest that environmental metals may contribute to amyloidogenesis, however, further in vivo and in vitro studies are needed to confirm such an outcome.

1032
INHIBITION OF HUMAN SQUALENE MONOXYGENASE BY SELENIUM COMPOUNDS.

N. Gupta and T. D. Porter. Graduate Center for Toxicology, University of Kentucky, Lexington, KY. Sponsor: M. Voss.

Excessive selenium exposure leads to a variety of toxicities in animals and men. However, the chemical species of selenium and the molecular targets that mediate its toxicity are not well characterized. Methyalted metabolites of tellurium, which is located directly below selenium on the Periodic Table, are potent inhibitors of squa- lene monooxygenase, the second enzyme in the committed pathway for cholesterol biosynthesis, and lead to a peripheral demyelinating neuropathy similar to that seen in selenium. To evaluate the toxicity of selenium and its methylated metabolites, we
examined the ability of selenite and three methylenediamine compounds, methyl- 
ethylenediamine, and trimethylammonium to inhibit purified recombinant human squamous non-cytotoxic. Selenite proved to be the most potent inhibitor of this enzyme, with an IC50 value of 37 μM; methyl-ethylenediamine (95 μM) and dimethylthalamine (680 μM) were less effective inhibitors, and inhibition by trimethylammonium iodide was evident only at concentrations above 3 mM. The inhibition by methylselenite and selenite was slow and irreversible, but activity could be restored by the addition of monothiols (glutathione, β-mercaptoethanol), indicating that the selenium compounds were reacting with sulfhydryl groups on the protein. Unexpectedly, the inhibition by selenite was signif- 
ificantly enhanced by diethanol (diaminoethane) diiodide, indicating that a more reactive group, possibly selenite, was formed in the presence of these diethanol reductions. These results raise the possibility that inhibition of squam- 
eous non-cytotoxic by selenite, selenite, and methylselenite may contribute to the 
neurotoxicity of selenium. Supported by a grant from the American Heart Association.

1033 INCREASED EXPRESSION OF PERIPHERAL BENZODIAZEPINE RECEPTORS IN THE MOUSE BRAIN FOLLOWING CUPRIZONE-INDUCED DEMYELINATION.
M. K. Chen and T. B. Guile. Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.

The peripheral benzodiazepine receptor (PBR) has been used as a sensitive marker of giacin and inflammation associated with neurotoxicity. Previous reports have suggested that PBR expression following brain injury is specific to areas expressing activated glial cells. Cuprizone, a copper chelator, has been used as a neurotoxicant to induce brain demyelination in mice. In the present study, we used this model in order to broaden the application of PBR as a biomarker of neurotoxicity, and to validate the relationship between PBR expression and glial cells. Adult male C57BL/6J mice were continuously fed a 0.2% (w/w) cuprizone-added powdered diet and sacrifi- ced after 2 and 4 weeks of treatment. [3H-(8)-PK11195 autoradiography] was per- formed to quantitatively measure PBR levels in anatomical regions. Increased expression of PBR was present in most white matter and in some gray matter regions of the cuprizone-exposed brain. Increased PBR levels were a function of the duration of cuprizone treatment. Consistent with previous studies on cuprizone neurotoxicity and pathology, significant (p<0.05) at 4 to 6 mg/kg, were noted in both frontal cortex (117%), hippocampus (114%), temporal cortex (106%), and entorhinal cortex (98%), as compared to controls. A greater number of regions and higher levels of PBR binding (p<0.01) were measured at 4 weeks of treatment. These included: cerebellar peduncles (290%), corpus callosum (237%), intermediate white matter superior colliculus (100%), globus pallidus (97%), thal- amus (82%), cerebellar deep nuclei (47%), striatum (389%), hippocampus (256%), and different areas of the cerebral cortex (226-332%). Present studies are assessing the glial cell type contributing to the increased levels of PBR in this model of demyelination. These studies support the notion that PBR can be a useful marker to visualize and measure neuropathological changes in brain following treatments that result in demyelination. (Supported by grant ES07602 to TRK)

1034 URANYL ACETATE-INDUCED SENSITIZATION DEFICIT AND INCREASED NITRIC OXIDE GENERATION IN THE CENTRAL NERVOUS SYSTEM OF RATS.

In the present study, we investigated the effects of uranyl acetate as a surrogate for DU on sensorimotor behavior, generation of nitric oxide and the central cholinergic system of rats. Male Sprague-Dawley rats were treated with 1.1 mg/kg i.p. injection of 0.1, and 1 mg/kg uranyl acetate in water, daily for 7 days. The animals were maintained on treatment free for an additional observation period of 30 days. On day 30 following the cessation of the treatment, the sensorimotor functions were evaluated using a battery of tests that included measurement of postural reflexes, limb placing, grip time, beam walking and incline plane performance. The levels of nitric oxide as marker for increased oxidative stress, and the integrity of the cholinergic as reflected in acetylcholinesterase activity and m2 muscarinic acetylcholine receptors ligand binding, in brain regions were determined. The data from behavioral observations showed that there was a dose-related deficit in incline plane performance. Both doses significantly reduced grip time. Similarly, both beam walk score and beam walk times were impaired at both doses as compared with the controls. A significant increase in nitric oxide was noted at 0.1 mg/kg dose in cortex and midbrain whereas brainstem and hypothalamus showed a decrease at both the doses. There was a significant increase in acetylcholinesterase activity in the cortex of the animals treated with 1 mg/kg uranyl acetate, but not in other brain regions. Ligand binding densi- ties for the m2 muscarinic receptor did not show any change. These results show that low-dose, multiple exposure to uranyl acetate, causes prolonged neurobehavioral deficits after the initial exposure has ceased, and suggest that exposure with uranium/DU may cause long-term neurological deficits.

1035 MOLECULAR MECHANISMS OF CHEMICAL TERATOGENESIS.
P. G. Wells I, T. J. Henderson, S. Krapivinova, R. R. Laposka, T. Parma, M. J. Wisny and L. M. Wim. I. Faculty of Pharmacy, Univ. of Toronto, Toronto, ON, Canada and 2. Dept. of Pharmacology, Univ. of Toronto, Toronto, ON, Canada.

Although over 50 years have passed since the thalidomide tragedy left thousands of infants born with severe malformations, only recently have there been a variety of biochemical and molecular biological approaches begun to reveal the underlying mechanisms and risk factors for chemical teratogenesis. This symposium provides a timely update of four approaches which, while diverse in technical repertoire and mechanistic thrust, all focus upon teratological mechanisms in the conceptus, providing both corroborating results and novel insights. First are the roles of reactive oxygen species-mediated signal transduction and oxidative macromolecular damage in teratogenesis, and the contributions of embryonic antioxidative enzymes and DNA repair in mediating teratogenesis. The second speaker focuses upon embryonic cellular thiol and redox-regulated developmental processes in sensitive and resist- ant species. The third approach investigates the teratological significance of alteration in gene expression and signal transduction pathways regulating conceptus cell cycle checkpoint activation, DNA repair and apoptosis. The last speaker focuses on the signaling pathways modulating apoptotic cell death in the developing embryo, and how their perturbation by xenobiotics may lead to teratogenesis.

1036 REACTIVE OXYGEN SPECIES (ROS) AND OXIDATIVE DAMAGE IN TERATOGENESIS.
P. G. Wells, T. J. Henderson, S. Krapivinova, R. R. Laposka, T. Parma, M. J. Wisny and L. M. Wim. 1. Faculty of Pharmacy, Univ. of Toronto, Toronto, ON, Canada and 2. Dept. of Surgery, Univ. of Toronto, Toronto, ON, Canada.

Using xenobiotics (phenytoin, thalidomide, BENZODIAZEPINE (BZN?)) and gamma irradiation, we examined the teratological relevance of embryonic bioactivation by prosstaglandin H synthase (PHS), ROS-mediated oxidative macromolecular target damage and signal transduction, and putative DNA repair in mouse, rat and rabbit models. The xenobiotics were bioactivated by purified PHS to free radical intermediates. PHS-2 expression was high in mouse embryos during organogene- sis; xenobiotic embryopathy was reduced by PHS inhibitors and in PHS-1 and PHS-2 knockout mice. Xenobiotic bioactivation, DNA/protein oxidation and emb- ryotoxicity occurred in embryo culture. In vivo embryonic DNA oxidation was re- duced to baseline within 24 hr, indicating active DNA repair. Treatment with antioxi- dative enzymes increased embryonic antioxidative activity, blocked DNA oxidation and inhibited teratogenesis, as did free radical trapping agents and antioxidants. Thalidomide embryonic DNA damage occurred only in susceptible (rabbit) but not resistant (species) animals. Hydroxyl radicals were formed in vitro, and teratogenecity increased either with mutant mice deficient in the antioxidative enzyme glucose-6-phosphate dehydrogenase, GSH depletion, or inhibition of GSH peroxidase and GSH reductase. Nitric oxide synthases (NOS) likely con- tribute to ROS-mediated teratogenesis, since NO knock out mice were partially protected. Inhibition of ROS reduced embryotoxicity, implicating ROS-mediated signal transduction. DNA may be a teratologically important target for oxidative lesions, since亚m and p53 knockout mice were more susceptible to teratogenesis, in some cases independent of apoptosis. These studies suggest that embryonic processes regulating the balance of ROS signaling, oxidative DNA damage and repair may be important determinants of teratological risk. (Supported: CIHR)

1037 REDOX-SENSITIVE MISREGULATION OF LIMB OUTGROWTH BY THALIDOMIDE.
G. Harris, J. M. Hames, E. W. Cramer, S. G. Gong and M. A. Phillips. Toxicology Program, Department of Environmental Health Sciences, Univ. of Michigan, Ann Arbor, MI, 1. Dept. of Orthodontics & Pediatric Dentistry, Univ. of Michigan, Ann Arbor, MI and 1. Health & Toxicology Research Laboratory, Dow Chemical Co., Midland, MI.

The ability of thalidomide to become bioactivated and generate free radicals and/or reactive oxygen species has been shown in several developmental and disease mod- els. Differences in the glutathione (GSH)/redox status and regulation in limb cells
from resistant (rat) and sensitive (rabbit) species suggest that redox regulation may be important in mechanisms of thalidomide-induced limb reduction defects. Exposure of whole animals, tissues and cultured limb cells show that the rabbit limb has lower overall levels of GSH and cysteine and cannot maintain a reducing nuclear redox environment when exposed to thalidomide. Limb outgrowth was regulated by NF-kB, a redox-sensitive transcription factor. No differences exist between sensitive and resistant species in terms of the ability of thalidomide to activate NF-kB in the cytosol and translocate it to the nucleus. The rabbit, however, is unable to maintain a reducing nuclear environment, not allowing NF-kB to bind its promoter and initiate limb outgrowth. Whole mount in situ hybridization of rabbit embryos exposed to thalidomide in utero with probes directed to Egr-1 twist and Ifg-8 show that thalidomide blocks or attenuates the expression of these limb outgrowth genes as a result of the inability of NF-kB to bind its nuclear promoter. NF-kB-mediated gene expression and limb outgrowth is restored by the addition of phenylbutylinone, a free radical trap. These processes are not affected in the resistant rat. Our studies show how environmental factors and chemicals can interfere with endogenous gene expression to result in developmental disease and malformation. Selective redox-misregulation of gene expression may be a critical factor in the mechanisms of developmental abnormalities produced by thalidomide and other chemicals capable of eliciting oxidative stress. (Support: OVPR-University of Michigan)

1038 HOW DOES THE CONCEPTUS RESPOND TO GENOTOXIC STRESS?

Many teratogens, either directly or via reactive oxygen species, increase DNA damage in the conceptus. Mammalian cells have evolved complex genotoxic stress responses to detect DNA damage and maintain genomic integrity, including DNA repair, cell cycle arrest, and apoptosis. We hypothesize that the ability of the conceptus to respond to genotoxic stress is a determinant of the outcome of teratogen exposure. Our first goal was to elucidate the expression profile of DNA repair/stress response genes following organogenesis in the rat. Second, we determined the consequences of exposure to a DNA damaging teratogen, 4-hydroperoxycyclophosphamide (4-OHCPA). Gestational day (GD)10-12 rat conceptuses were analysed with the ArNA Technique. Transcripts for 17 nucleotide excision repair (NER) genes, the major mammalian DNA repair pathway, were expressed at low levels on GD10, except for XPD, XPE and PCNA. There was stage dependent increase in XPB expression in yolk sac and in LH23B, XBP, XPE, ERCC1, and DNA polymerase epsilon expression in the embryo on GD12. Whereas 4-OHCPA induced malformations, it did not affect NER transcript levels. Except for UNG, APNG, PMSI, and RAD54, genes in the base excision repair (BER), mismatch repair (MMR) and recombination repair (RCR) pathways were not detected or expressed at low levels on GD10. Interestingly, UNG transcripts (BER) increased from GD11-12 by over 400%. Unlike the NER genes, 4-OHCPA down-regulated transcripts for most BER, MMR, and RCR genes in the embryo. The ATM stress-response pathway regulates cell cycle arrest after DNA damage. While in yolk sac ATM expression was constant from GD10-12, in the embryo ATM transcripts increased dramatically (>500-fold) on GD12, 4-OHCPA decreased ATM expression in the embryo but increased it in yolk sac. Thus, DNA repair and stress response gene expression in the conceptus is regulated in a pathway-dependent, developmental stage- and tissue-specific manner. Understanding how the embryo responds to genotoxic stress will assist in the design of strategies to protect the conceptus from insult. (Support: CHIR & FCAR)

1039 APOPTOTIC SIGNALING PATHWAYS IN TERATOGENESIS.
P.E. Miranda. Dept. of Pediatrics, Univ. of Washington, Seattle, WA.

Between 2 and 3% of all live-born infants have a major developmental defect identified at birth. Although the etiology of these birth defects is often unknown, environmental exposures are known to play a significant role. One of the early events in the pathogenesis leading to birth defects, induced by a wide variety of environmental agents, is cell death. The first part of my talk will describe our research showing that teratogens such as hyperthermia, cyclophosphamide, sodium arsenite, and staurosporine all induce an early episode of cell death in exposed day 9 mouse embryos. This cell death is apoptotic in nature and is characterized by the release of mitochondrial cytochrome c followed by activation of a caspase cascade. Thus, these 4 teratogens induce cell death by activating the intrinsic, mitochondrial apoptotic pathway. Teratogen-induced cell death is also selective, i.e., some cells in the embryo die while the majority of cells do not. For example, we have shown that cells of the developing CNS are particularly sensitive to teratogen-induced cell death whereas cells of the developing heart are completely resistant. The second part of my talk will present more recent data showing that this heart cell resistance

1040 OLFACTORY TRANSPORT OF INHALED METALS: AN IMPORTANT ROUTE OF DELIVERY TO THE BRAIN.
D. C. Dorman and J. R. Hakimian.

CJIT Centers for Health Research, Research Triangle Park, NC and Department of Food Safety and Toxicology, Michigan State University, East Lansing, MI.

The olfactory system is unique in that it forms a direct interface between the air and the central nervous system (CNS). There is growing evidence that metals deposited within the nose can be absorbed at this site and then undergo transport along the olfactory nerve. The transport of metals within the olfactory nerve can result in direct delivery of inhaled metals to the brain. In some cases, direct olfactory transport is the dominant pathway by which an inhaled metal reaches the CNS. This symposium will discuss what is known about the neuronal transport of metals in the olfactory pathway and will include a discussion of the likely mechanisms by which transport occurs. This symposium will also cover clinical features of metal-induced nasal toxicity in humans and will discuss interspecies differences in nasal anatomy that may play a role in metal olfactory uptake and transport. The metal of special concern to be discussed in this symposium is manganese, a neurotoxic metal shown to be able to cross synapses in the olfactory bulb and migrate via secondary olfactory neurons to distant nuclei of the brain.

1041 COMPARATIVE NASAL STRUCTURE, FUNCTION AND TOXICOLOGY: RELEVANCE TO OLFACTORY TRANSPORT OF METALS.
J. R. Hakimian.

Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI.

In laboratory animals, several metals (e.g., Al, Cd, Mn, Ni, Zn) pass via olfactory neurons from the nasal airway lumen to distant sites in the brain. Exposure to metal-containing compounds (e.g., NiSO4, ZnSO4) may also cause marked toxicity of the olfactory mucosa. Differences in the nature and magnitude of metal-induced responses (physiologic or pathologic) of the olfactory mucosa to inhaled metals among mammalian species may be due to species-specific differences in nasal airway structure and function. The purpose of this introductory lecture is to provide a concise review of the similarities and differences in nasal anatomy, physiology, and toxicologic pathology among laboratory animals and humans, emphasizing important comparative aspects of the olfactory mucosa. Rodents are obligate nose breathers and appreciate highly odorous compounds by olfactory neuroepithelium with direct neural connections to the olfactory bulb. In contrast, primates are oral breathers with only a small amount of their nasal lumens lined by olfactory epithelium. These species differences along with differences in nasal airflow only translate into differences in intranasal deposition of inhaled metals and subsequent metal transport to the brain and/or olfactory toxicity. In addition, injury, adaptation and repair of the olfactory epithelium may markedly alter metal transport to the brain. Knowledge of comparative nasal structure, function and toxicity is essential for interpreting animal data and estimating the potential risks of nasal or neurological damage in humans exposed to inhaled metals.

1042 METAL TRANSPORT IN THE OLFACTORY SYSTEM.

We have examined the transport of several metals (manganese, cadmium, nickel, mercury, zinc and cobalt) in the olfactory system of rats and mice. Among these metal ions, manganese has been found to have a unique capacity to be taken up via the olfactory pathway. Thus, following transport along the primary olfactory neurons to the olfactory bulb, manganese continues via other neurons to all parts of the brain and even into the spinal cord. Studies with nickel, zinc and cobalt indicate that these metals also pass from the primary to the secondary olfactory neurons. Cadmium and mercury are transported along the primary olfactory neurons but appear unable to continue along secondary olfactory neurons. Studies in mice indicate that cadmium and manganese move along the primary olfactory neurons by fast axonal transport, whereas nickel moves by slow axonal transport. We assume that the metals will adhere to proteins or other endogenous neuronal con-
Olfactory Transport of Inhaled Manganese.

D. C. Dorman. GIFT Centers for Health Research, Research Triangle Park, NC.

Research efforts examining the dosimetry of inhaled manganese primarily focus on pulmonary deposition and subsequent hemagogenous delivery of the metal to the central nervous system. Growing evidence suggests that nasal deposition and transport along the olfactory bulb and the telencephalon via transport in secondary olfactory neurons. Until recently, little was known regarding olfactory transport of manganese following inhalation exposure. We have conducted studies in rats using short-term (90-min) inhalation exposure to radiolabeled manganese aerosols (54Mn, or 54Hf). Studies using an animal model in which one nostril was occluded thus prohibiting olfactory transport of manganese to one side of the rat brain. We found that the olfactory route contributes the majority (>70%) of the 54Mn found in the olfactory pathway of the brain following acute inhalation exposure. However, neither of these inhalation studies clearly demonstrated that olfactory uptake contributes significantly to increased striatal manganese concentrations. Results of 14-16 inhalation studies in rats exposed to relatively soluble or insoluble manganese aerosols further demonstrate that manganese concentrations achieved in the olfactory bulb are significantly higher than that observed in either the striatum or cerebellum, lending credence to the direct olfactory transport hypothesis. The relevance of these findings to human manganese inhalation exposure and the risks for neurotoxicity are not known and are complicated by interspecies differences in nasal and brain anatomy and physiology. In the rat, the olfactory bulb accounts for a relatively large proportion of the central nervous system, and the nasal olfactory mucosa covers approximately 50% of the total nasal epithelium. These structures are proportionately smaller in humans, suggesting that this route of brain delivery may be less important in humans as compared to the rat. These differences likely predispose the rat, more so than humans, to olfactory deposition and potential olfactory transport of manganese.

Nasal Toxicity of Metals in Humans.

F. W. Sunderman, Jr.1, 2Middlebury College, Middlebury, VT and 2University of Vermont, Burlington, VT.

Occupational exposures of workers to inhalation of certain metal dusts or aerosols can cause diverse forms of nasal toxicity, including loss of olfactory acuity, atrophy of the nasal mucosa, perforated nasal septum, or sinonasal cancer. Anosmia and hyposmia have been reported in workers exposed to Ni- or Cd-containing dusts in alkali battery factories, nickel refineries, and cadmium industries. Anosmia has also developed in children following experimental nasal spraying with zinc sulfate solution. Ulcers of the nasal mucosa and perforated nasal septum have been reported in workers exposed to Cr(VI) in chromate production and chrome plating, or to As(III) in arsenic smokers. Cancers of the nose and nasal sinuses have been reported in workers exposed to Ni compounds in nickel refineries, cutlery factories, and alkali battery manufacture, or to Cr(VI) in chromate production and chrome plating. In humans, the olfactory bulb contains an abundance of certain metals (Cd, Mn, Hg) compared to other regions of the brain, which is consistent with experimental evidence in animals that metals can enter the brain via the olfactory tract.

Introduction: The Unfilled Promise of Biomarkers.


The 1983 NRC publication formalized human health risk assessment into a four-component process: material exposure assessment, hazard identification, dose-response assessment, and risk characterization. Since that report, research has focused on addressing scientific uncertainties within each of these components. Less emphasis has been given to research to strengthen the linkages between these elements to better understand the health effects associated with acute human exposures. The emergence of biomarkers of inhalation exposures in current studies demonstrates that it is not novel to consider such measures in establishing biologically significant pathways and/or potential health risks. However, the report does provide a timely opportunity to reconsider the scientific status and opportunities for biomarkers and to reconsider their role in the context of the current and future scope of the CDC program, a case history will be presented that uses blood lead to demonstrate the attributes of a successful biomarker. The potential role of pharmacokinetic modeling to link exposure and effects biomarkers will be considered followed by an examination of the challenges to toxicology and epidemiology to provide the research that led to the development of such biomarker data similar to that being generated by CDC. This abstract has been submitted to Agency for review and approval for publication.

CDC's Biomonitoring Program: The National Report on Human Exposure to Environmental Chemicals.

E. J. Sampson. Division of Laboratory Sciences, Centers for Disease Control and Prevention, Atlanta, GA. Sponsor: H. Zenick.

The Centers for Disease Control and Prevention (CDC) has established a National Report on Human Exposure to Environmental Chemicals that provides biomonitoring data from participants in the National Health and Nutrition Examination Survey (NHANES). In the Report, CDC measured 27 chemicals in blood and urine samples from this ongoing national survey of the United States population. The chemicals included metals (e.g., lead, cadmium, mercury, uranium), carbon monoxide, and phthalate metabolites. Highlights of the Report show that blood lead levels among children have declined since the early 1990s and that there has been a major reduction in exposure to the US population to environmental tobacco smoke. In addition, the Report provides new data on children's and women's exposure to mercury and lead in the general population to 27 chemicals. The Report will be updated each year with new data for the general population. For example, next year CDC will combine the 1999 survey data with new data from participants in NHANES 2000 to provide updated national estimates on the 27 chemicals. Plans to expand the report with additional chemicals each year; eventually exceeding 100 chemicals measured on the population per year. Chemicals considered under consideration for future reports include volatile organic compounds, carcinogenic polynuclear hydrocarbons, diols, furans, polychlorinated biphenyls, carbamate pesticides, and organochlorine pesticides. Future additions to the Report will provide more detailed assessments of exposure levels among different population groups defined by sex, race/ethnicity, age, urban/rural residence, education, income, and other characteristics.

Blood Lead Levels as Biomarkers and Surveillance Indicators.

E. K. Silverberg. Program on Human Health and the Environment, University of Maryland, Baltimore, MD.

The National Health and Nutrition Examination Survey (NHANES) is a periodically conducted nationally representative surveillance of the US population. Its data on national blood lead levels in the US population have been among the most successful biomarkers for environmental health ever undertaken. In NHANES II (conducted from 1976 to 1980) the first national prevalence data on lead exposures revealed the extent of excess lead exposures and the socio-demographic determinants of environmental racism related to lead. These data were influential in the campaign to remove lead from gasoline, culminating with the 1990 amendments to the Clean Air Act. These data have also stimulated attempts at reducing sources of lead exposure in housing and urban environments. The NHANES III (1991-1994) demonstrated the effectiveness of these policy interventions by showing significant reductions in blood lead levels among all subgroups of the US population (age, gender, ethnicity, income, and residence), but these data also showed the continued disparities in lead risks for US children under 6 years. NHANES data have also been used to generate important hypotheses on lead toxicity. Using NHANES data, studies have associated lead with decreased growth in stature, deficits in hearing, and increased blood
pressure. These data have also suggested a potential role for menopause in increasing blood lead. These blood lead data are powerful for two reasons: (1) the surveillance program is carefully designed for national relevance, and (2) toxicology allows us to draw inferences between the biomarker and health risks. Thus, we understand biomarkers collected in well designed studies can be extraordinarily useful in both research and public policy.

**1048 BIOMARKERS AND PHARMACOKINETIC MODELING: THE KEY TO ESTABLISHING THE EXPOSURE–DOSE-EFFECTS LINKAGE.**

M. L. Rigas, H. A. Barton and M. S. Okino. National Exposure Research Laboratory, USEPA, Las Vegas, NV and NHEERL, USEPA, Research Triangle Park, NC.

Measures of chemical in tissues, blood, or urine are useful to the extent that they can be related to health effects and because they are an integrated measure of absorbed dose following exposure. However, the direct relationship between biomonitoring data and pathway-specific exposures is more tenuous. Pharmacokinetic (PK) models are mathematical representations of, at minimum, absorption, clearance and can estimate internal dose based on multi-route exposure. The reverse calculation to extract a unique exposure profile based on biomonitoring data is not possible. PK models can provide useful information relating biomonitoring data to exposure using additional data such as those generated by human exposure field studies to aid in these estimates. There are several methods that use PK models and biomarker data to further understand the timing and magnitude of exposures. In one case, we have explored this relationship using the urinary metabolite of the organophosphate insecticide chlorpyrifos collected as part of a pesticide exposure study in children. To set bounds for the modeling, we assumed that the children received some constant low-level background exposure and were subjected to intermittent higher level exposure events as a consequence of pesticide application in the home. Using a PK model and a mathematical algorithm to constrain possible exposure scenarios based on urine data of pesticide usage by the families, we calculated plausible exposure scenarios that would lead to the urinary biomonitoring results. It is also important to consider how the pharmacokinetic properties (e.g., elimination half-life) of a compound are related to the certainty bounds of the exposure estimation from biomarker data. (The USEPA, through its Office of Research and Development, funded this research. The abstract has been subjected to Agency review and approved for publication.)

**1049 IMPLICATIONS OF BIOMARKER DATA FOR THE FUTURE OF TOXICOLOGY AND RISK ASSESSMENT.**

J.S. Bus, Toxicology & Environmental Research & Consulting, The Dose Chemical Company, Midland, MI.

The CDC’s Annual Human Exposure Report will provide valuable exposure information impacting the future design and interpretation of toxicology studies, and also assure continued improvements in the integration of this toxicology and human exposure data into risk assessments. Data describing human burden of chemicals will be pivotal inputs to future dialog surrounding potential expansion and/or alteration of conventional toxicology testing protocols to more reasonably mirror real-world exposures (e.g., implications for use of traditionally defined MTDs in toxicity hazard testing design of mixture studies, etc.). However, the Annual Report would be of even greater value to the debate if it were expanded to include analyses of natural products. Such comparative perspectives (synthetic vs. natural) could significantly inform future directions of risk assessment, e.g., what represents appropriate Uncertainty Factors in Margin-of-Exposure based risk assessments. The Annual Report also will provide data critical for effective validation and refinement of human exposure models, improving their ability not only to more accurately estimate potential exposures resulting from manufacturing, distribution, and use of chemicals, but also to more effectively measure the efficacy of regulatory and/or product stewardship decisions. Despite the enormous potential of the Annual Report, efforts to augment the future of toxicology and risk assessment, there must be concurrent emphasis that such biomonitoring data does not by itself indicate risk, i.e., it is the dose, not just the presence, that makes the poison.

**1050 DRUG-INDUCED VASCULAR DISEASE: MARKERS OF INJURY.**


Injury to vascular structures is a relatively common hazard identified in preclinical drug toxicity studies. The understanding of action of novel compounds which cause vascular injury do not always fit accepted dogma that relates cardiovascular damage to marked hypotension and reflex tachycardia. Terminology, relative to classification of vascular lesions by pathologist and clinicians, also presents communication issues since the term vasculitis evokes differing images, mechanisms and consequences to clinicians of differing backgrounds. Historically, studies in animals have not predicted the drug-induced necroses observed in humans, nor have the drug-induced vascular lesions recognized in animals been associated with clinical consequences. Collectively, these issues culminate in confusion when attempting to predict potential hazards of new drugs in a clinical setting and emphasize the need for non-invasive markers that can be used preclinically and clinically to monitor for development of drug-induced vascular injury. The lack of markers for vascular injury has caught the attention of toxicologists, pathologists, vascular biologists, radiologists and clinicians. This dilemma has prompted significant investigation, with novel approaches, to identify circulating markers predictive of damage to endothelial cells or cells of the vascular wall. Investigations have employed a spectrum of antibody-based, flow-cytometric assessments or magnetic resonance spectroscopy-base technologies on various body fluids and tissues as well as traditional plasma-based evaluation of endothelial products. The workshop will be briefly discuss the possible approaches for detection of vascular injury, the terminology to facilitate common understanding and communications and to have in-depth discussions of new markers, technical approaches and their promise for future application.

**1051 PLASMA MARKERS OF VASCULAR INJURY: NEW TOOLS FOR THE TOXICOLOGIST AND CLINICAL PHARMACOLOGIST.**

A. Blann. City Hospital NHS Trust, Birmingham, United Kingdom. Sponsor: W. Kemps.

Damage to the endothelium is thought to be important in the initial clinical stages of atherosclerosis and in the onset of several connective tissue diseases. Consequently, we have had the opportunity, in a clinical setting, to correlate changes in certain plasma markers with vascular disease and its progression. Among the plasma markers evaluated, reliable, sensitive, and specific markers of endothelial function in humans include von Willebrand factor, soluble E selectin and soluble thrombomodulin. Each represent different aspects of the physiology and cell biology of the vessel wall, the pathophysiology of the disease process and development of clinical events. Measurement of these markers by ELISA has helped provide essential contributions to our understanding of the development of cardiovascular disease as well as in risk factors (diabetes, hypertension, smoking) and in cancers and the diseases related to rheumatoid arthritis such as systemic sclerosis and systemic lupus erythematosus. For example, increased concentrations of some of these markers predict a poor prognosis such as myocardial infarction and stroke. Notably, von Willebrand factor can also be measured easily in rats, but it's value as a possible marker for vascular damage is unknown. Therefore, as the markers have predictive power in helping identify those persons at risk for development or progression of disease, it seems possible that they may prove useful in studying vascular injury in cases of acute (aspirin) and chronic (heavy metals) poisoning. Following a brief review of endothelial cell biology, the predictive nature of various markers will be established from case studies in humans.

**1052 USE OF METABONOMIC TECHNOLOGY TO NON-INVASIVELY ASSESS DRUG-INDUCED VASCULAR INJURY.**

D. G. Robertson. Drug Safety Evaluation, Pfizer Global Research & Development, Ann Arbor, MI.

As a class, the vasculitides encompass a variety of preclinical and clinical conditions and are, in general, empirically defined and poorly understood because of the lack of specificity of associated signs and symptoms, the inaccessibility of target tissues for pathologic examination, and importantly, the absence of efficient non-invasive diagnostic tests. Metabonomics is a recently emerging approach that combines high-resolution nuclear magnetic resonance spectroscopy (NMR) of biologics with pattern recognition technology to evaluate the metabolic status of an organism. The underlying principle of metabonomics, as with traditional biomarkers of disease, is that homeostatic alterations during disease processes or toxic insult may be reflected directly or indirectly in body fluids, and may ultimately leave biochemical traces in urine. The advantage of metabonomics lies in its ability to monitor simultaneously and non-invasively tremendous numbers of trace molecules. A metabonomic evaluation of urine collected from animals given model compounds has demonstrated the utility of this technology to detect changes induced by drugs that cause vascular injury. Appropriate discretion is advised as the technique is sensitive to unrelated pharmacologic and clinical effects that can complicate interpretation of the NMR spectra and the challenge remains to link specific changes with specific types of vascular injury. Nonetheless, this technology has shown significant promise for sensitively and selectively identifying animals with vascular injury by analysis of
Studies in many instances, the neoplasia has been attributed to disruption of normal physiology. When this disruption is sustained, a series of well-characterized changes occur in the target organ including hypertrophy, hyperplasia and eventually neoplasia. The ovary (granulosa cell), thyroid (follicular cell), and stomach (endocrine cell) were selected because sufficient investigative data exist to characterize the mode-of-action for several compounds. In addition, sufficient understanding and experience exist to provide context for their relevance to human risk assessment. Each speaker will describe the physiology of the target organ and provide examples of mode-of-action by which compounds disrupt physiologic function. A primary focus will be discussing the comparative differences of structure and function of these selected target organs between rodents and humans, as well as relating the findings to human endocrine disease states and epidemiology to assess whether these non-genotoxic mechanisms of neoplasia are relevant to human risk assessment.

1056 SECONDARY MECHANISMS IN THYROID CARCINOGENESIS

R. M. McClain, Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ.

Numerous studies have reported that chronic treatment of rodents with goitrogenic compounds results in the development of tumors derived from thyroid follicular cells. Many goitrogenic xenobiotics increase the incidence of thyroid tumors in rodents by a direct effect on the thyroid gland to disrupt one of several steps in the biosynthesis, secretion, and metabolism of thyroid hormones. This includes (a) inhibition of the iodine trapping mechanism, (b) blockage of organic binding of iodine and coupling of iodothyronines to form thyroxine (T4) and triiodothyronine (T3), and (c) inhibition of thyroid hormone secretion by an effect on processes of active thyroid hormone from the thyroid. Another large group of goitrogenic chemicals disrupt thyroid hormone economy by increasing the peripheral metabolism of thyroid hormones through an induction of hepatic microsomal enzymes. This group includes CNS drugs, calcium channel blockers, steroids, redox, chlorinated hydrocarbons, polyaromatized hydrocarbons, and enzymes inductors. Thyroid hormone economy also can be disrupted by xenobiotics that inhibit the 5-monoxygenase that converts T4 in peripheral sites to biologically active T3, inhibition of this enzyme by iodine-containing compounds such as FD&C Red No. 3 lowers circulating 13 levels, which results in a compensated increased secretion of thyroid stimulating hormone (TSH), follicular cell hyperplasia, and an increased incidence of follicular cell tumors in rats. Physiologic perturbations alone, such as the feeding of an iodine-deficient diet, partial thyroectomy, natural goitergens in certain foods, and transplantation of TSH-secreting pituitary tumors in rodents also can disrupt thyroid hormone economy and, if sustained, increased the development of thyroid follicular cell tumors. A consistent finding with all of these goitrogens is the chronic hypersecretion of TSH which places the rodent thyroid gland at greater risk to develop tumors through a secondary (indirect) mechanism of thyroid oncogenesis associated with hormonal imbalances. The human thyroid is much less sensitive to this pathogenetic phenomenon than rodents.

1057 MECHANISMS OF HORMONE-MEDIATED CARCINOGENESIS OF THE OVARY

C. C. Capen, Department of Veterinary Biosciences, The Ohio State University, Columbus, OH. Sponsor: J. Cook.

The objectives of this presentation are to: 1) discuss selected examples of non-genotoxic endocrine mechanisms of ovarian carcinogenesis in rodents, and 2) consider the relevance of rodent ovarian tumor development for human risk assessment. Factors that destroy or greatly diminish the number of ovarian follicles in rodents include senescence, genetic deletion of follicles. X-irradiation, a wide variety of marketed drugs and xenobiotics, transplantation of ovaries into the spleen, and early thyroectomy with the development of autoimmune antibodies to oocytes. All are known to diminish sex steroid hormone secretion by the ovary. Other mechanisms include xenobiotics that bind to the estrogen receptor in the hypothalamus. (e.g. selective estrogen receptor modulators such as Raloxifene), genetic deletion of the estrogen receptor (ER)-α (ER knock out mice), and transgenic expressing chimeric luteinizing hormone (LH)-B subunit. The resulting elevated circulating levels of gonadotropins, especially (LH), due to disrupted feedback on the hypothalamus-pituitary axis operators ovarian tumor development in rodents. For example, the long-term-receptor-mediated stimulation of stromal (interstitial) cells by LH and indirectly the ovarian surface epithelium places the mouse ovary at increased risk for developing unique tubular (tubulo)stromal adenomas. The formation of similar complex ovarian tumors in genetically similar mice not exposed to xenobiotic substances, supports the prevention of tumor formation by estrogen administration or implantation of normal ovaries supports a secondary (hormonally mediated) mechanism of ovarian oncogenesis. The intense proliferation of ovarian surface epithelium and stromal cells with frequent development of complex
ovarian tumors in rodents as a response to sterility does not have a counterpart in the ovaries of women and is not a useful predictor for the development of ovarian neoplasia.

RODENT LEYDIG CELL TUMORIGENESIS AND THE RELEVANCE TO HUMAN HEALTH.


Leydig cells (LCs) are the cells of the testes which have as their primary function the production of testosterone, and are a common target in rodent carcinogenicity bioassays. To date, 7 mechanisms for the chemical induction of Leydig cell tumors (LCTs) have been elucidated. Most of these mechanisms involve, ultimately, elevation in serum luteinizing hormone (LH). In contrast to the rat, LH does not appear to be a primary driver of LCTs in the mouse. In the mouse, estrogen receptor antagonists/agonists are the key to the development of these tumors due to their direct mitogenic response at the LC. Hence, the induction of LCTs in mice does not appear to be relevant to humans due to the differences in the hypothalamus, pituitary, and testis. However, pathways for regulation of the hypothalamic-pituitary-testis (HPT) axis of rats and humans are similar, such that compounds which either decrease testosterone or estradiol levels or their concentration will increase LH levels. Although, there are several lines of evidence which suggest that human LCs are quantitatively less sensitive than rats in their proliferative response to LH, and hence, in their sensitivity to chemically-induced LCTs. This evidence includes the following: (1) the human incidence of LCTs is much lower than in rodents; (2) several comparative studies have demonstrated differences exist between rat and human LCs which may contribute to the greater susceptibility of the rat to both spontaneous and xenobiotic-induced LCTs; (3) the endocrine disease states in man underscore the marked comparative differences that exist between rats and man in the responsiveness of their LCs to proliferative stimuli; and, (4) several human epidemiology studies are available on the relationship between exposure to compounds that induce LCTs in rats which demonstrate no association between human exposure to the chemicals and any increase in incidence. Overall, the evidence suggests that nongenotoxic compounds which induce LCTs in rats may have low relevance to humans because humans are quantitatively less sensitive than rats to the mitogenic effects of LH.

NGENOTOXIC MECHANISMS OF EXOCRINE PANCREATIC CARCINOGENESIS IN RODENTS AND THEIR RELEVANCE TO HUMAN HEALTH.

D. S. Longnecker. *Pathology, Dartmouth Medical School, Lebanon, NH.* Sponsor: J. Cook.

Spontaneously arising neoplasms in the exocrine pancreas of the rat are almost exclusively acinar cell tumors. Most genotoxic carcinogens that affect the pancreas induce acinar cell neoplasms that are histologically similar to spontaneous tumors. Several nongenotoxic dietary additives including trypsin inhibitors and peroxotene proliferators, as well as high fat diets, have increased the incidence of acinar cell neoplasms in the pancreas of rats. Many of these also promote the development of neoplasms in animals pretreated with genotoxic pancreatic carcinogens. It is likely that the nongenotoxic dietary additives are promoting the growth of spontaneously-initiated clones of acinar cells which are manifest as focal hyperplasia in "control" rats. The mechanism of the promoting effect of high fat diets is not known, however promotion by trypsin inhibitors and cholesic agents has been linked to an increase of cholecystokinin (CCK) secretion. CCK serves as a mediator of enzyme secretion and as a trophic factor for the pancreas in rats. Injection of exogenous CCK stimulates pancreatic growth (hyperplasia) and promotes carcinogenesis in carcinogen-treated animals. The feedback loop that stimulates excess CCK has been delineated and linked to intraluminal inhibition of tryptic activity or diminished bile secretion. In human fewer than 1% of pancreatic carcinomas are of acinar cell type and about 90% have a ductal phenotype. Exocrine secretions are largely under neural control in humans, and it is uncertain that CCK is trophic for human pancreas. Some data suggest that human acinar cells express few receptors for CCK. These species differences raise a question as to the relevance of CCK-mediated promotion mechanisms in the human. Furthermore, there is no epidemiological evidence linking ingestion of trypsin inhibitors to increased risk of pancreatic carcinoma.

GASTRIC ENTEROCHROMAFFIN CELL-LIKE (ECL) CARCINOIDS IN RODENTS AND THEIR RELEVANCE TO HUMAN DISEASE.


Clinically, carcinoids develop as a component of atrophic gastritis or as part of the multiple neuroendocrine neoplasia (MEN)-1 syndrome (both types due to hypergastrinemia) or as sporadic lesions. Agents that cause hypergastrinemia and stimulate ECL cell proliferation have been investigated in a number of conventional models (rat, mouse) as well as the Mastomys and Japanese cotton rat. Pharmacological doses (100 x human levels) of H2 receptor antagonists or proton pump inhibitors result in ECL cell hyperplasia (8-10 wks) and eventually neoplasia (>2yr) in rats, mice and cotton rats. A number of agents commonly found in the human diet (methyliodin, used as anti-oxidant or in crop preservation (butachlor) are all tumorigenic and cause ECL carcinoids in rodents; effects due to gastric mucosal damage and hypergastrinemia. In contrast, in the Mastomys, the standard therapeutic doses of an acid suppressive agent result in ECL cell hyperplasia (6-8 wks) with tumor formation by 4 months. Tumor development in this rodent and in mice is not a number of common traits. Carcinoids may spontaneously develop over the life-time of both species. These tumors are somatostatin receptor type 2 (SSTR2) positive. Somatostatin (octreotide) therapy prior to acid perturbation inhibits tumor formation in this animal, while in vitro studies demonstrate that this may affect both ECL cell secretion as well as negatively regulate hyperplasia. Human and Mastomys tumors also share alterations in growth factors (TGF-alpha), in c-myc (overexpressed) and in the mitogen (seismic mutations). One difference is in the ECL cell growth response to acid suppression; a doubling of ECL cell numbers is seen in humans, while neoplasia is most often seen in Mastomys. The underlying genomic alteration (s) is unknown. The substantial overlap in tumor pathobiology between the Mastomys and the human suggest that this rodent may specifically be an important model for investigating nongenotoxic gastric carcinoid formation.

ATH RECEPTOR ACTIVATION REQUIRES INDUCTION OF MAP KINASE PATHWAYS BY TCDD.

Z. Tan, X. Chang, A. Puga and Y. Xia. *Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH.*

TCDD is a widespread environmental contaminant that causes adverse toxic effects in animals and humans. Toxicity is mediated by activation of the cytosolic aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor essential for TCDD-induced expression of several phase I and phase II drug metabolizing enzymes. TCDD also activates signal transduction pathways, but the molecular mechanisms by which these pathways may contribute to AhR activation and TCDD toxicity have yet to be defined. Here we report that TCDD induces in mouse hepatoma Hepa-1 cells the same set of extracellular signal-regulated kinases (ERKs) and Jun N-terminal kinases (JNKs), two subgroups of the mitogen-activated protein kinase (MAPK) family involved in modulation of transcription factor activity and regulation of gene expression. Our results show that MAPK activation by TCDD is not mediated by the AhR, since it takes place in CV-1 cells and in AhR(-) mouse embryonic fibroblasts, both of which lack AhR expression. Unlike treatment with serum factors or tumor promoters, TCDD-stimulated MAPK activities did not result in the transcriptional activation of Elk and c-JUN but was critical for the induction of AhR dependent gene transcription. Chemical compounds that inhibit ERK and JNK/p38 pathways and dominant negative effectors of the ERK pathway suppressed induction by TCDD of AhR-dependent reporter gene expression by Majano et al. (1994) expression was also partially suppressed by inhibitors of the ERKs or the JNKs pathways. Inhibition of CYP1A1 induction was maximal when both pathways were blocked, suggesting that the ERK and the JNK pathway each make unique contributions to AhR activation. These results indicate that induction by AhR ligands of MAPK pathways is an important contributor to AhR activation of gene expression. (Supported by NIH 2RO1-ES6273 and 1RO1-ES10807).

BRG-1 IS REQUIRED FOR CYTOCHROME P450A1 TRANSCRIPTION MEDIATED BY THE ARYL HYDROCARBON RECEPTOR COMPLEX.

S. Wang and O. Hamilton. *Pathology, UCLA, Los Angeles, CA.*

Chromatin remodeling is a key step in overcoming the nucleosomal repression of active transcription in eukaryotes. The mammalian SWI/SNF ATP-dependent chromatin-remodeling complexes contain multiple subunits. The major ATPase activities in these complexes are attributable to either BRG-1 or the related Brahma (Brm) protein. The aryl hydrocarbon receptor (AhR), after binding xenobiotic ligands such as 2, 3, 7, 8-terachlorodibenzo-p-dioxin (TCDD), associates with the AhR nuclear translocator (ARNT), and the dimer activates transcription of several genes, including cytochrome P450A1 (CYP1A1). We show that introduction of BRG-1 into the BRG-1 and Brm deficient SWI3 and C33A human cell lines enhances expression from a transiently transfected AhR/ARNT-dependent reporter gene. We also demonstrate that replenishment of BRG-1 to SWI3 cells restores endogenous cytochrome P450A1 (CYP1A1) expression, whereas an ATPase deficient mutation of BRG-1 is unable to do so. Using CHIP (Chromatin Immunoprecipitation), we demonstrate that BRG-1 associates with the enhancer region of the mouse CYP1A1 gene in vivo in a TCDD- and ARNT-dependent fashion, suggesting the specific recruitment of BRG1 by AHR/ARNT. Finally we demonstrate that the transcriptional activation domain of AHR interacts with
BRG-1. Together our studies reveal an essential involvement of BRG-1 in activating CYP1A1 gene transcription and implicate the importance of ATP-dependent chromatin remodeling activity in inducible gene expression mediated by AHR/ARNT.

1063 IDENTIFICATION OF HSP90 CHAPERONE COMPONENTS THAT REGULATE ARYL HYDROCARBON (DIOXIN) RECEPTOR SIGNALING.
C.A. Miller and M. B. Cox. Environmental Health Sciences, Tulane University Health Sciences Center, New Orleans, LA.

The chaperones are a family of highly conserved proteins that play numerous roles in cells. Among these roles, Hsp90 proteins and their co-chaperones are proposed to regulate the conformation and function of nuclear hormone receptors. Aryl hydrocarbon receptor is a transcription factor that is activated by xenobiotic ligands such as TCDD and requires interactions with Hsp90 proteins for proper function. We constructed a yeast model system that used transcriptional activation by the human aryl hydrocarbon receptor-Anti receptor complex as an endpoint to genetically assess which chaperones and co-chaperones interact with the signaling pathway. Most Hsp90 chaperones and co-chaperones are structurally and functionally conserved between yeast and humans, thus making this a relevant model system for these studies. Genetic evidence from strains lacking particular chaperone and co-chaperone genes indicates that the proteins Hsp82 (a homolog of human Hsp90) and Hsl1 (a homolog of human p23), and the tetratricopeptide repeat (TPR)-containing proteins Cpr6 and Cpr3 have roles in the aryl hydrocarbon receptor signaling pathway.

The absence of other Hsp90 and co-chaperone proteins (Hsp82, Cpr6, Ptp1) had no effect in this assay. Thus, specific chaperone and co-chaperone proteins participate in the aryl hydrocarbon receptor signaling pathway. We propose that the cellular levels and interactions of chaperones and co-chaperones may determine the response to TCDD and other compounds that act through the aryl hydrocarbon receptor.

1064 IDENTIFICATION OF DOMAINS REQUIRED FOR ARYL HYDROCARBON RECEPTOR INTERACTION WITH THE RETINOBLASTOMA PROTEIN AND FOR INHIBITION OF EARLY-DEPENDENT TRANSCRIPTION.
J. L. Marlowsky, J. K. Kerker, E. S. Knudsen and A. Puss. Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH.

TCDD is a tumor promoter and carcinogen in rodents, and epidemiological evidence suggests a role in the development of breast cancer in humans. Our previous research has suggested that the mechanism of TCDD's ability to activate AHR may involve the formation of a heterodimeric complex with the retinoblastoma protein (Rb). The Rb tumor suppressor gene is a tumor suppressor gene that is frequently inactivated in breast cancer and is thought to play a role in regulating cell cycle progression. To determine the role of the various Rb proteins in AHR-mediated transcription, we used full-length Rb cDNAs. We found that Rb proteins containing an Rb domain that interfered with the Rb interaction delayed the formation of the Rb/AHR complex.

1065 ERK ACTIVATION BY GROWTH FACTORS OR EXPRESSION VECTORS RESTORES TCDD-SENSITIVITY IN UNRENEWED-SEUM ADIPOCYTE DIFFERENTIATION OF C3H10T/1/2 CELLS.
P. R. Hannon and G. L. Marks. Environmental Toxicology, University of Wisconsin, Madison, WI.

C3H10T/1/2 mouse embryo fibroblast cells differentiate into adipocytes when treated with insulin, dexamethasone, methylisobutyrate, and the PPARY ligand BRL49653 (IDM/BRL). In the presence of fresh fetal bovine serum (FBS), PPARY expression and adipocyte differentiation are inhibited by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). However, with unrenwed FBS adipocyte differentiation is TCDD-insensitive. Addition of Epidermal Growth Factor (EGF) had no effect on PPARY expression or adipocyte differentiation in the absence of TCDD. However, in the presence of TCDD, EGF restored inhibition of adipocyte differentiation in a dose-dependent manner. On the other hand, Fibroblast Growth Factor partially inhibited PPARY expression and adipocyte differentiation in the absence of TCDD, and also restored TCDD-sensitivity in a dose-dependent manner. Addition of specific pharmacological inhibitors of growth factor receptors during serum stimulation showed that no single growth factor within FBS was responsible for the TCDD-sensitivity. Pharmacological inhibitors of EGF activation show that the TCDD-mediated inhibition of adipocyte differentiation is dependent on ERK activation in a nutrient time window. Addition of a MEK1 dominant positive vector to cells in unrenwed FBS also restored TCDD-sensitivity to the process of adipocyte differentiation. These data establish that factors from an ERK-activated pathway act through a TCDD-activated pathway work synergistically to inhibit PPARY expression and adipocyte differentiation.

1066 DISRUPTION OF NEPHROGENESIS BY BENZOA(I)/PYRENE, AN ARYL HYDROCARBON RECEPTOR LIGAND, INVOLVES INTERFERENCE WITH ALTERNATIVE SPlicing AND POST-TRANSCRIPTIONAL REGULATION OF THE WtMLS TUMOR SUPPRESSOR 1 GENE.
M. Falahatpisheh and K. S. Ramos. Center for Environmental and Rural Health, Texas A&M University College Station, TX.

The aryl hydrocarbon receptor (Ahr) is a member of the basic helix-loop-helix and PAS homology domain family of transcription factors. Members of this family are involved in the regulation of neurogenesis and myoblast differentiation. Unregulated activation of Ahr signaling is associated with hydrocarbon exposure, but the mechanisms responsible for this response are unknown. The present studies were designed to evaluate the influence of benzo[a]pyrene (BaP) on murine nephrogenesis in vitro. Embryonic kidneys were dissected from E11.5 mouse embryos and placed in humid culture chambers. Decreases rates of glomerulogenesis and deficits of cellular differentiation were observed in cultures challenged with 3 μM BaP for 4 days. BaP interfered with podocyte differentiation and identification of glomerular basement membranes. Ahr and Wtms1 tumor suppressor (WT1) mRNA levels were detected by in situ hybridization and reverse transcription polymerase chain reaction (RT-PCR) in differentiated, as well as BaP-treated metanephric cultures. Using specific primers for WT1 (a) WT1 splice variant it was found that the appearance of the (c) WT1 splice variant, although total WT1 mRNA levels were unchanged by BaP treatment. Immuno localization experiments showed a significant decrease in WT1 protein immunoreactivity in cultures challenged with BaP compared to control counterparts. Western analysis and visualization of this finding and identified a unique WT1 isoform in metanephric cultures treated with BaP. Comparison of metanephric cultures from Ahr+/+ and Ahr−/− showed that Ahr is required for kidney development, as well as BaP-induced deficits in nephrogenesis. Consequently, these results indicate that activation of Ahr signaling by BaP disrupts nephrogenesis in vitro by modulation of alternative splicing and post-transcriptional regulation of the WT1 gene. (This work was supported in part by NIH grants ES 04917 and ES 09106).

1067 AHR REPRESSOR: A THIRD MEMBER OF THE VERTEBRATE ARYL HYDROCARBON RECEPTOR (AHR) FAMILY.

The effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds on the AHR, a member of the bHLH-PAS protein superfamily. A single AHR gene has been identified in mammals, while many fish species, including the Atlantic killifish (Fundulus heteroclitus) possess two distinct AHR genes (AHR1 and a novel form, AHR2). A bHLH-PAS protein closely related to AHR was recently identified in mouse and designated AHR repressor (AhR) (Mimura et al., Genes Dev 1999). Mouse AhR is induced by 3-methylcholanthrene and represses the transcriptional activity of the AHR. The sequence similarity between AHR and AhR is relatively low and the presence of a single AHR ortholog in Fundulus and identified a cDNA with high sequence identity to the mouse and human AHRR. Fundulus AhR encodes a 686 residue protein with a predicted

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molecular mass of 75.2 kDa. Phylogenetic analysis shows that AHRs (mammals and fish) form a third clade within the AHR family, along with AHR1 (mammals and fish) and AHR2 (fish only); these three vertebrate AHRs diverged from a single invertebrate AHR. Thus, mammalian AHR is not the ortholog of fish AHR2. We show here that in vitro-expressed AHR proteins from human, mouse, and Fundulus all fail to bind [3H]TCDD or [3H]-beta-naphthoflavone. In transient transfection experiments in COS-7 cells using a DRE-luciferase reporter gene, Fundulus AHR had transactivation activity, which could not be blocked by TCDI-dependent transactivation by both AHR1 and AHR2. Fundulus AHR mRNA is widely expressed and is inducible by TCDD or PCBs. The Fundulus AHR promoter contains three putative dioxin-responsive elements. Both AHR1 and AHR2 activated transcription of luciferase driven by the AHR promoter, and AHR2 could repress this transactivation. Thus, AHR is an evolutionarily conserved, TCDI-inducible repressor of AHR1 and AHR2 function. [NIH Superfund ES07381 and NIH ES06272]

1068 GRIP1 ENHANCES AHR SIGNALING IN HEPA1C1C7 CELLS.
S. R. Rushing, C. L. Jones and M. S. Dennis. Environmental Toxicology, UC Davis, Davis, CA.

Exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds produces a variety of tissue and organ specific toxic and biological effects, most of which are mediated via the aryl hydrocarbon receptor (AhR) signaling pathway. The AhR is a ligand-dependent transcription factor that regulates gene expression via its binding to the dioxin responsive element (DRE) as a heteromeric complex with the AhR nuclear translocator (ARNT) protein. The events necessary for transcriptional activity by the DNA-bound AhR complex remain to be elucidated. As part of our effort to identify nuclear factor(s) which can modulate the transcriptional activity of the AhR complex, we have examined the ability of the glucocorticoid receptor interacting protein 1 (GRIP1) cofactor to affect AhR/ARNT-dependent signal transduction. GRIP1, like the AhR and ARNT, contains a basic helix-loop-helix/Per/DBP domain located near the N-terminal and two transcriptional activation domains, AD1 and AD2, near the C-terminal. Co-transfection of Hepa1c1c7 cells with a DRE-reporter construct and a GRIP1 expression plasmid results in a 5-8 fold increase in reporter activity over cotransfection with the empty parent vector alone. However, when these cells were cotransfected with a GRIP1 lacking AD1, reporter activity was not enhanced, suggesting a role for AD1 in the augmentation of AhR signaling. Co-immunoprecipitation and GST pull-down techniques revealed strong interactions between GRIP1 and the AhR in at least two regions. The addition of GRIP1 also enhanced AhR/ARNT/DNA complex formation as assessed by gel retardation analysis. These results demonstrate that GRIP1 acts as a coactivator of the nuclear AhR complex. Supported by NIEHS ES070972, ES057070 and ES046999.

1069 CROSS-TALK BETWEEN THE ARYL HYDROCARBON RECEPTOR AND SIGNAL TRANSDUCTION PATHWAYS: A ROLE FOR NF-kB.
E. M. Khan and M. S. Dennis. Environmental Toxicology, University of California, Davis, CA.

Activation of gene expression by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and related chemicals is mediated by the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor. Previously, a role for protein kinase C (PKC) in AhR signal transduction was demonstrated, with PKC inhibiting AhR functionality and phosphorylating 12-13-acetylated (PMA)-dependent PKC activation enhancing AhR-dependent gene expression. In the present study we have extended these analyses and demonstrate that the PMA enhancement of AhR signaling in mouse hepatoma (Hepa1c1c7) cells is dependent upon the AhR DNA-binding site and translocation, occurring within the first eight hours after PMA treatment. While the enhancing effect appears directly related to gene transcription, the inhibition of PKC is associated with decreases in nuclear AhR and gene expression. PMA is also a potent activator of nuclear factor-kB (NF-kB)-dependent reporter gene expression in these cells. Not only do these chemical inhibitors of NF-kB (pyrrolidindithiocarbamate (PDTC), (E)-capsaicin (CAPS), and caffeic acid phenethyl ester (CAPE)) block PMA-induced expression from an NF-kB reporter gene, but they inhibit both normal and PMA-enhanced AhR-dependent gene expression. Although gel retardation analysis reveals that inhibition of PKC by chelerythrine chloride reduced AhR nuclear translocation, NF-kB inhibition by PDTC had no effect on nuclear AhR levels. These results demonstrate a role for both PKC and NF-kB in the regulation of AhR-dependent gene expression. (Supported by NIEHS grant ES07685)

1070 TNF-α TREATMENT SUPPRESSES CYP1A1 TRANSCRIPTION BY INHIBITING ACETYLATION OF HISTONE H4 AND PHOSPHORYLATION OF THE C-TERMINAL DOMAIN OF RNA POLYMERASE II.
Y. Tan,1 A. B. Rabson3, M. A. Gallo1 and S. Ke1, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX. 2Environmental and Comparative Medicine, EOHSI-UMDNJ, Piscataway, NJ and 3CABARCIN, UMDNJ, Piscataway, NJ.

It has been known that TNF-α and lipopolysaccharide (LPS) suppress the gene expression of cytochromes P450 1A1 (cyp1a1). In earlier studies, we demonstrated that activation of NF-kB is a critical event leading to the suppression of cyp1a1 gene expression, thus establishing a cross-interaction between the Ah receptor and NF-kB signal transduction pathways. In the present study, we demonstrated that Ah receptor/NF-kB interactions converge at level of transcription involving two steps of transcriptional regulation: (1) chromatin remodeling and (2) transcriptional elongation by RNA polymerase II (RNA Pol II). Specifically, using cyp1a1 transcription in Hepa1c1c7 cells as a model system, we demonstrated that dioxin treatment (10 nM, 2 hrs) causes significant histone H4 acetylation which is associated with chromatin remodeling activity. TNF-α treatment (5 ng/ml) markedly suppresses this histone acetylation activity, especially around the TATA box region, suggesting that NF-kB activation inhibits the histone H4 acetylation at cyp1a1 promoter. Furthermore, we demonstrated dioxin treatment causes phosphorylation of the C-terminal domain (CTD) of RNA Pol II, a critical step of transcriptional elongation. TNF-α treatment significantly suppresses the dioxin-induced RNA Pol II phosphorylation, especially at serine 2 of YSPTSPS motif of the RNA Pol II CTD. These results establish a mechanism for the inflammatory cytokine-induced inhibition of cytochrome P450 1A1, suggesting the involvement of chromatin remodeling and transcriptional elongation in the “cross-talk” between Ah receptor and NF-kB signaling pathways. Supported in part by NIEHS Grant ES09859 and NIEHS Center Grant ES05022.

1071 MECHANISM OF HORMONAL ACTIVATION OF EARLY GROWTH RESPONSE-1 (egr-1) IN BREAST CANCER CELLS.
C. C. Chen and S. H. Safe. Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.

Egr-1 is induced by 17β-estradiol (E2) in estrogen receptor (ER)-positive MCF-7 breast cancer cells, and analysis of the egr-1 gene promoter indicates that there are multiple cis-elements that could be activated by ER through genomic and non-genomic pathways. The E2-responsive -650 to +12 region of the promoter does not contain an estrogen response element (ERE), and direct binding of ER to the egr-1 promoter has not been detected. 5’ Deletion analysis of the promoter showed that the -460 to -164 region of the promoter was essential for ER-responsiveness, and this sequence contains 5 serum response elements (SREs) and two Ets motifs. Identification of E2-responsive motifs was further investigated using a series of deletion constructs (egr-1:1) containing the -640 to -283, -385 to -283, and -460 to -385 regions of the egr-1 gene promoter, respectively. In transient transfection studies in MCF-7 cells, E2 induced reporter gene activity in cells transfected with pgrf-1 (-460 to -283) (7.5-fold), and only weak induction (-2-fold) was observed in cells transfected with pgrf-1E (-385 to -283). The remaining constructs were not hormone-responsive suggesting that the upstream SRE is the major site of E2-induced transactivation. These results are similar to those recently reported for non-genomic activation of the proximal SRE in the e-gr1 gene by E2 and, in MCF-7 cells transfected with pgrf-1E, hormone-induced activation was blocked by the MAPK inhibitor PD98059. Current studies are focused on characterizing the SRE-binding transcription factors and the pathway for E2-dependent MAPK activation of Egr-1. (Supported by NIH ES09253 and ES09106)

1072 IN VIVO STRESS ACTIVATES JAK-STAT IN LIVER BUT NOT BRAIN.
D. B. Miller, S. A. Benkovic and J. D. O’Callaghan. Toxicology & Molecular Biology, CDCN/OSIH, Morgantown, WV.

The Janus kinase-signaling transducers and activators of transcription (JAK-STAT) signaling pathways are believed to be crucial signaling pathways in the physiological changes induced by stress. Various stressors (e.g., UV) have been shown to activate the JAK-STAT pathways, in vitro, but only a few reports have examined the JAK-STAT pathways using stress models, in vivo. Here C57BL/6j female mice were exposed to restraint, vibration or cold stress. To preserve steady-state phosphorylation, the mice were killed by focused microwave irradiation. The activation status of STAT 3, 5 and 6 was assessed from blots of tissue homogenates probed with phospho-state-specific antibodies as well as antibodies directed against the context-independent state of the various STATS to assess their total tissue levels. Quantification
was achieved by densitometry of bands generated by enhanced chemiluminescence. No change in the activation (phosphorylation) state of any of the STATs were evident in brain; however, large increases in phosphoSTAT3 were evident in liver, with restraint and cold providing the largest magnitude changes (~300%). STAT3 levels did not change with any of the stressors. Restraint of adrenalectomized mice caused an even larger increase in phosphoSTAT3 (~900%). Immunohistochemical examination of STAT3 confirmed that restraint resulted in the nuclear location (i.e., activation) of STAT3, which was substantially greater in adrenalectomized mice. These data suggest that effects of physiological stressors are mediated through a STAT3 pathway that may involve the HPA axis and/or sympathetic nervous system.

COSTS AND BENEFITS OF COMPLIANCE WITH ALTERNATIVE REMEDIATION STANDARDS AT HEXAVALENT CHROMIUM-CONTAMINATED SITES.

P. D. Williams and D. Presthus. Exponent, Menlo Park, CA.

The New Jersey Department of Environmental Protection (NJDEP) requires varying levels of soil remediation at hazardous waste sites depending on the pathway of exposure. For example, sites which contain hexavalent chromium (Cr(VI)) in soil must comply with either uniform default or site-specific health-based standards for inhalation, dermal, and oral exposures. NJDEP currently relies on an "averaging" approach when evaluating the increased cancer risk from inhalation of suspended soil (i.e., the 95th percentile above upper confidence limit of the mean concentration is not allowed to exceed the inhalation standard) and a "bright line" approach when evaluating allergenic contact dermatitis (ACD), an acute effect, from direct contact with Cr(VI) in soil or puddles (i.e., an acute effect standard). NJDEP also relies on this latter "bright line" approach when evaluating the long-term non-cancer risks associated with incidental soil ingestion, although this is inconsistent with USEPA risk assessment guidelines. The current analysis evaluates whether the extra costs required to comply with NJDEP's approach are justified by the associated benefits. Cost-benefit estimates are made on average costs for site remediation and the difference in the volume of soil requiring treatment to meet the oral standard as either a "bright line" or an "average." Benefits are estimated as the expected change in health risk associated with NJDEP's approach, and assumptions about the population affected and value per fatal and non-fatal event averted. The results of the cost-benefit analysis indicate that NJDEP's approach for site remediation yields negative net benefits—i.e., estimated costs are about $100,000 per site examined as compared to estimated benefits of about $100. Sensitivity analyses, which incorporate a range of possible costs and benefits, also suggest that NJDEP's approach is not cost-beneficial. These findings are important for evaluating the appropriateness and limitations of alternative approaches for complying with soil remediation standards.

INVESTIGATION OF ASBESTOS IN CONSUMER PRODUCTS: CHILDREN'S PLAYSANDS.


The US Consumer Product Safety Commission (CPSC) regulates 15,000 types of consumer products, including products made with mined materials. Recently, CPSC staff obtained a convenience sample of six play sand products from three companies in five states. Under contract, two independent laboratories analyzed the sand by polarized light microscopy (PLM) and transmission electron microscopy (TEM) for the presence of asbestos fibers. The bulk of each sample was nonfibrous and consisted of quartz, with smaller amounts of rutilite, sphene, zircon, hornblende, rock fragments, and opaque grains. Two samples from the same company contained chrysotile and tremolite asbestos (<0.001%). Fibers were small, about 1 μm long and 0.05-0.2 μm in diameter. Small chamber experiments were conducted to determine if asbestos fibers could be released into the air. Air fibers were analyzed by TEM using the USEPA AHERA (Asbestos Hazard Emergency Response Act) protocol. Estimated asbestos fiber release from the two play sand samples was 210, 000-2.5 million fibers per minute. Airborne fibers had the same mineralogy and physical characteristics as fibers detected in the bulk samples. Thus, despite the relatively small proportion of asbestos structures in the sand, large numbers of fibers were released into the air during a simulated play activity. Indoor air modeling was based on one hour of play in a daycare or classroom setting in projected air concentrations of 0.03 to 0.35 fibers/mL. Although the staff concludes that the very short fibers detected in these samples are not a health concern, we will continue to monitor play sand and other mineral-containing consumer products for the presence of potentially hazardous asbestos fibers. (The opinions expressed by the authors do not necessarily represent the views of the Commission. This abstract is in the public domain and may be freely copied or reprinted.)

DERMAL ABSORPTION OF PCBs IN RHESUS MONKEYS FROM SOIL CONTAMINATED WITH AROCLOL 1260.


Dermal absorption is routinely evaluated as a potential pathway for absorption of contaminants when conducting human health risk assessments for Superfund sites. For Aroclor 1260-contaminated soil, the US Environmental Protection Agency uses a dermal absorption factor of 14%, a factor derived from a 1993 study of the dermal absorption of Aroclor 1262 and 1254 from contaminated soil by Rhesus monkeys. In comparison to the 1993 study, the current study varied several parameters that can influence the dermal absorption of lipophilic hydrocarbons, including soil organics content, soil particle size, skin residence time and contaminant "aging" in the soil. The study consisted of four groups of four female rhesus monkeys exposed to 14C-labeled Aroclor 1260. One group was exposed intravenously to PCBs in propylene glycol (100% absorption) and 3 groups were exposed dermally to PCB-spiked soil. Two dermal exposure groups were exposed for 12 or 24 hours, respectively, to PCBs that had been aged in soil. These groups exhibited percutaneous absorption of 3.43 ± 0.35% and 4.26 ± 0.52%, respectively, of the applied dose. The remaining dermal group was exposed for 24 hours to soil freshly spiked with PCBs and exhibited dermal absorption of 4.07 ± 0.46% of the applied dose. It has been reported that soil organic content is an important factor in modulating the percutaneous absorption of highly lipophilic compounds from soil. The soil used in the current study had an organic content of 5-6%, a value typical for US soil, and a value that contrasts sharply with the 0.9% soil organic content used in the 1993 study upon which the current 14% dermal absorption factor for PCBs is based. Therefore, the current dermal absorption factor used by EPA appears to substantially overestimate the fraction of PCBs available for absorption from soils with typical organic content.

EPAS INTEGRATED RISK INFORMATION SYSTEM (IRIS).


IRIS is an EPA database that contains consensus scientific positions on potential human health effects that may result from chronic exposure to chemical substances. IRIS assessments present summaries of quantitative and qualitative health effects information, including reference doses (RfDs) and reference concentrations (RfCs) for the noncarcinogenic effects of chemicals and cancer slope factors and unit risks. Quantitative assessments in IRIS are used extensively in combination with specific situational exposure assessment information to evaluate potential public health risks. The IRIS database currently contains assessments for over 500 chemical substances. Approximately 80 assessments are underway or will be initiated in FY 2002; these include assessments for chemicals new to IRIS and updates of existing summaries that provide new scientific information and application of more current risk assessment methodologies. The IRIS Program continues to explore ways to improve its assessments, the process by which the assessments are prepared, and the database itself. Two such initiatives are the re-engineering of the IRIS Web site and an IRIS "Needs Assessment." The Web site (www.epa.gov/iris) was redesigned to be a more user-friendly Web-enabled database, with extensive hypertext and search interfaces. Users can request a "QuickView" version, which provides essential values with links to full IRIS summary text and supporting documents. The Needs Assessment is intended to define the needs of the public and EPA program offices/regions for new and revised IRIS health assessments. Issues being examined include: what additional chemical substance assessments are needed, which assessment currently on IRIS are in greatest need of scientific update, what additional types of information would be of value (e.g., acute and subchronic values); and to what extent should EPA collaborate with external parties as a means of developing IRIS assessments. Input from this Needs Assessment will be used by EPA in developing the 2002-5 IRIS agenda and resource needs.

EVALUATION OF NON-CARCINOGENIC EFFECTS OF SOLUBLE NICKEL SULFATES.


A risk assessment for chronic toxicity of soluble nickel (reference dose, RfD) stress exposure was prepared by USEPA in 1987 and is available on EPIs Integrated Risk Information System (IRIS). The RfD was based on a two year feeding study in rats given 0, 100, 1000, and 2500 ppm nickel in the diet (estimated as 0.5, 50, and 125 mg/kg/day). No significant effects were reported at 100 ppm (5 mg Ni/kg/day). At 1000 ppm, body weight was significantly reduced in both sexes. At 1000 ppm, males had significantly higher heart-to-body weight ratios and lower liver-to-body weight ratios than controls. The two year survival was poor, particularly in control

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rats, both males and females (84% and 92% moratility, respectively), raising some concern about the interpretation of the results of this study. Using NOAEL of 5 mg/kg/day and an uncertainty factor of 300 (10 each for intraspecies variability and 3 for lack of conclusive reproductive toxicity data) the RfD calculated was 0.002 mg/kg/day. A subchronic study conducted by American Biogenics Corporation, also found 5 mg/kg/day to be a NOAEL. Recently Springborn Laboratories completed a two generation reproductive study in which rats received 0, 1, 2.5, 5.0 or 10 mg/kg/day nickel sulfite hexahydrate (0.0, 0.22, 0.56, 1.12 or 2.22 mg Ni/kg/day) by gavage. Based on the absence of adverse effects on fertility/fecundity, fetal/neonatal survival and on developmental measures at any of the doses tested, the NOAEL identified in this study was 10 mg nickel sulfite hexahydrate/kg/day (2.2 mg Ni/kg/day). Increased fetal loss was observed at higher doses in the range finding study. Using this NOAEL and an uncertainty factor of 100/10 for intraspecies variability the RfD calculated is 0.002 mg/kg/day which is similar to the one currently on IRIS. The opinions expressed are those of the authors and do not reflect USEPA policy.

1078 RE-EXAMINATION OF THE PROPOSED ARSENIC (AS) DRINKING WATER REGULATION.

In 2001, the EPA extended the effective date for the 1982/83 Drinking water rule until 2/22/01. This enabled EPA to conduct an extensive review on the health effects of As and the costs/benefits of the rule. In addition, EPA asked the National Research Council (NRC) to consider health data published since its 1999 report entitled Arsenic in Drinking Water. The Agency also asked the National Drinking Water Advisory Council (NDWAC) to review EPA’s cost calculations and the Science Advisory Board (SAB) to suggest methods to improve benefits analysis. The 2001 NRC panel concluded that recent data suggests that the +3 As metabolites might directly react with DNA and that the risks for bladder and lung cancer could be greater than the estimates in the earlier report. The NDWAC group reviewed the costing methodologies and assumptions underlying the system-wide cost estimates as well as the aggregated national estimate of system costs of the As Rule. They also evaluated other costing approaches and critiques that may have an impact on the system costs. They were charged to determine whether there is adequate information to evaluate the basis for the alternate approaches or critiques and note where there is not adequate supporting information. For the benefits estimates, the SAB panel asked EPA to consider the latency after initial exposure and the lag between reduction of exposure to As and reduction of risk for bladder and lung cancer. EPA should also quantify the benefits from prevention of some additional diseases, eg, diabetes mellitus, chronic heart disease, and skin cancer; increase its valuation of non-fatal diseases; and expand its uncertainty analysis. The Agency is weighing the risks of adverse health effects at different As exposures, the costs of treatments to meet the new As standard and the benefits from limiting As exposure in drinking water to concentrations of 3, 5, 10 or 20 µg As/L. EPA will use the balance among these three factors to set the new As Maximum Contaminant Level. [These opinions are the authors’ and not necessarily those of the EPA]

1079 TESTING FOR THRESHOLDS AND CALCULATING THRESHOLD DOSES WITH NONLINEAR DOSE-RESPONSE RELATIONSHIPS IN BIOASSAYS FOR CARCINOGENICITY.
W. K. Lutz and R. W. Lutz. Toxicology, University of Wurzburg, Wurzburg, Germany and Statistics, ETH, Zurich, Switzerland.

Nonlinear dose-response relationships are often postulated to be thresholded, but definition, statistical criteria, or estimations of threshold doses (TD) are usually lacking. We propose a procedure to estimate TD, based on a linear extrapolation of the line between the response at the lowest-observed-effect level (LOEL) and the non-observed-effect level (NOEL) to intersect with the dose axis. It is exemplified for bioassays for carcinogenicity with groups of 50 animals. The following data set was used: incidence 0.50 tumor-bearing animals (TBA) at dose 0; insignificant incidences of 1/50 to 5/50 TBA at dose 1; significant responses with 10, 15, or 20/50 TBA at dose 2. The assumption is that the observed sublinearity is a matter of chance only, in that the true incidence at dose 1 is higher than the observed incidence, while the true incidence at dose 2 is lower than the observed. True incidences were calculated by attributing a given percentage of a binomial distribution to the observed incidences. The symmetrical 80% confidence intervals were chosen, which result in a p=0.0, 1.0, 1.0. Linear extrapolation of the line that connects the true incidences at dose 1 and 2 resulted in the following dose levels at intersection with the dose axis: for 10/50 TBA at dose 2, incidence 1/50 at dose 1 resulted in an intersection at 0.1 dose units; for higher incidences at dose 1, the intersection was outside the threshold dose range of 0-1. For 15/50 at dose 2, incidences 1/50 and 2/50 at dose 1, but not higher, intersected at 0.60 and 0.30 dose units, respectively. For 20/50 at dose 2, thresholds were indicated for incidences up to 4/50, but not for 5/50 (TD) spanning from 0.75 to 0.17 dose units. In addition to the estimation of threshold doses (lower bounds), the procedure allows the estimation of a probable threshold that the true threshold is 1 and extrapolate linearly to the incidence in the controls. This could be regarded as a p-value for rejecting the null hypothesis of linearity, for an observed sublinearity.

1080 MULTIPLE DETECTION OF CYTOKINES AND PHOSPHORYLATED PROTEIN KINASES IN VITRO AND IN VIVO USING THE BIO-PEX PROTEIN ARRAY SYSTEM.

Cytokines and kinases play pivotal roles in cell signaling events, mediating a wide range of physiological responses. Here we present data from the Bio-Plex protein array system demonstrating the sensitive, simultaneous quantitation of 8 cytokines in human or mouse sera, and in tissue culture media. We can quantify all 8 cytokines at a limit of detection less than 10pg/ml, and with inter- and intra-assay CV less than 10%. Using an 8-plex assay, a single 96-well plate can generate 786 concordant cytokine values from 12 pl of sample/well. We also demonstrate a multiple sandwich immunosassay that simultaneously measures phosphorylation levels of JNK, p38MAPK, ERK, Akt and Jak2 in a single well of a 96-well plate. These high content, multiplexed assays are highly correlated with and as sensitive as ELISA and immunoblotting. All the assays are specific and exhibit no detectable cross-reactivity between antibodies. Most importantly, data obtained from multiplexed cytokine and phosphoprotein assays provide an invaluable profile of cellular response for drug discovery and development.

1081 BIOMARKERS IN HUMAN AND RAT: A PROTEOMICS APPROACH.
1082 RAPID METHOD FOR DETECTION AND IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN DRUG-TREATED CELLS.
T. T. Pham1, S. Fu1, W. Zhang1, J. H. Lin2 and S. Durham1, 1Cephergen Biostystems, Fremont, CA and 2Bristol-Myers Squibb Pharmaceutical Co, Princeton, NJ; Sponsor: O. Flink.

Cell lines, in response to several drug treatments, regulate the amount of proteins expressed within the cell as well as the secreted proteins to the cell media. We have used a ProteinChip System and the Reverse-phase Chromatography Mass Spectrometry method (RC-MS) to monitor differentially expressed proteins in the cell media. Many proteins were detected to be up- or down-regulated when proteins in cell media were profiled using several chromatographic ProteinChip Arrays. Two low abundant, small protein biomarkers are subsequently purified for protein identification. Based on their binding characteristics to the arrays, we have quickly developed protein enrichment and purification methods using microtitre plates together with a sensitive protein detection method by RC-MS assisted protein purification. We have identified these purified proteins at high confidence after tryptic digestion, peptide-mass mapping on the ProteinChip System and searching the publicly available protein databases. We show this technique as a new method to monitor the toxicological effect of drugs.

1083 IDENTIFICATION OF Ubiquitin-CONJUGATED PROTEINS USING LC-MS TECHNIQUES: POTENTIAL MOLECULAR INDICATORS OF TOXIC INSULTS.
D. S. Kirkpatrick, K. V. Dale, D. C. Liebler and A. J. Gambold, Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

Post-translational modification of proteins plays a key role in cellular stress responses and recovery. In mammalian cells, ubiquitin, the protein conjugated to many proteins for proteosomal degradation, is now implicated in a number of proteosome-independent signaling processes. In order to study responses in the ubiquitin pathway following intoxication, a method has been developed to screen samples for ubiquitin conjugates. For this work, liquid chromatography-mass spectrometry (LC-MS) methods have been combined with newly developed search algorithms to study ubiquitin-conjugated Histone 2A/2B. Insoluble fractions were prepared from HEK293 cells and separated by SDS-PAGE. Gel bands of molecular weight 22 kDa, known to contain ubiquitin-tagged Histone 2A and/or 2B, were excised and subjected to in-gel digestion with trypsin. MS-MS spectra were obtained from tryptic peptide mixtures on an LCQ-MS (ion trap). Peptide sequences matching both Histones 2A and 2B, as well as ubiquitin, were independently identified using SEQUEST and SILAS search algorithms to mine LCQ data files. Significant sequence coverage was obtained for Histone 2B (44%) and ubiquitin (34%), and to a lesser extent for Histone 2A (18%). Furthermore, the identification of one particular C-terminal ubiquitin peptide strongly suggests that certain peptides from ubiquitin-conjugated proteins carry a short polypeptide tag specifically identifying the modified residue. Thus far, MS-MS spectra have not yielded sequence coverage for peptides containing the lysine residue specifically modified by ubiquitin, probably because of complications arising from the modification that allow them to evade SEQUEST and SILAS detection. Despite this, Histones 2A and 2B have contributed significantly to our understanding of MS-MS fragmentation characteristics of ubiquitin-conjugated proteins and allowed us to search complex protein pools for molecular indicators of chemical intoxication. (Supported by NIH Grants ES 06694, ES 04940, ES 07091)

1084 IDENTIFICATION OF ORGANOPHOSPHATE INSECTICIDE TARGETS IN SH-SY5Y HUMAN NEOUROBLASTOMA CELLS.
K. M. George1, D. H. Williamson1, C. J. Burgess1, T. M. Voelker2 and C. M. Thompson1, 1Center for Environmental Health Sciences, University of Montana, Missoula, MT; 2Department of Pharmaceutical Sciences, University of Montana, Missoula, MT; and 3Department of Chemistry, University of Montana, Missoula, MT.

Organophosphate (OP) insecticides increase agricultural production, but are considered highly toxic to humans and animals due to inhibition of acetylcholinesterase (AChE) resulting in cholinergic hyperstimulation. OP’s have been implicated in many other pathological diseases, including Gulf War Syndrome, Parkinson’s disease, cancers, and various immune dysfunctions. Other esterases, such as neurotoxic esterase, have been shown to be inhibited by OP, but it is likely that OP’s interact with and modify the activity of other proteins. We have synthesized a biotinylated fluorophosphonate, FP-biotin, which shares common OP structural characteristics, but has a biotin group attached. Following covalent modification of the protein by FP-biotin, it is possible to visualize or isolate FP-biotin-bound proteins via avidin affinity. Our hypothesis is that OP compounds interact with neuronal cell proteins other than AChE. To test this hypothesis, we treated human neuroblastoma cells, SH-SY5Y, with varying concentrations of FP-biotin and tested for AChE inhibition and cell viability over time. Lysates were made of SH-SY5Y cells treated with FP-biotin and biotin-labeled proteins were purified using an avidin-affinity column prior to visualization by western immunoblotting. Biotinylated proteins were digested with trypsin either in-solution or in-gel and analyzed by automated nanoLC-MS/MS. Accurate peptide mass fingerprinting combined with peptide fragment analysis was used to identify additional OP-protein conjugates. These experiments demonstrate that OP compounds bind proteins other than AChE in neurons, and may identify complementary pathways to cholinergic hyperstimulation leading to pathology.

1085 UP AND DOWN REGULATION OF PHOSPHORYLATED HEPATIC PROTEINS FOLLOWING ADMINISTRATION OF THE PROTEIN PHOSPHATASE INHIBITOR, MICROCYSTIN-LR TO RATS.
L. D. Rigg, F. E. Regnier, C. R. Wilson and S. E. Hooper, Chemistry and Veterinary Pathobiology, Purdue University, West Lafayette, IN.

The field of proteomics is seeing an effort to utilize high throughput methods to quantify the degree in which cellular proteins change when exposed to disease, drugs, toxins, etc. Gallium(III) immobilized affinity chromatography can be used to select phosphorylated peptides. Affinity selected phosphopeptides can be analyzed by using phosphatase enzymes and mass spectrometry. Phosphopeptides are identified by comparing their mass/charge and sequence via MS/MS to databases. Quantification of the degree to which peptides and parent proteins are up- or down-regulated is achieved by determining relative concentrations of samples labeled with acetate and experimental samples with triethanolamine. Control and experimental samples are mixed, affinity selected and resolved as above. Peptides appear in the mass spectrum as doublets separated by multiples of 3 mass units. The isotope ratio of non-changing proteins is approx. 1:1. Those that have a different ratio have undergone some regulatory change. Microcystin-LR (MCLR) is an inhibitor of protein phosphatases 1 and 2A. However, the change in phosphorylation state is only known for a few proteins which have been specifically identified. In this study, male rats were treated with microcystin-LR (500 μg/kg, ip) or saline. At 6h, they were necropsied. Liver sections were fixed or frozen. Microscopic liver necrosis was typical of MCLR. Frozen liver samples were homogenized, digested with trypsin, affinity selected via gallium(III) immobilized affinity chromatography, and quantified via mass spec. Gall(III)-IMAC selection captured approximately 2800 phosphorylated peptides. MALDI-TOF mass spec of acetate-labeled peptides identified phosphoproteins which had undergone regulatory change. The majority were up regulated compared to controls, some were down regulated or unchanged. This suggests that the regulation of protein activity via phosphorylation is complex with both increases and decreases in phosphorylation following inhibition of protein phosphatases.

1086 SELDI-TOF MASS SPECTROMETRY PROTEOMIC ANALYSIS OF PYRIDOSTIGMINE BROMIDE AND STRESS ON THE HP-AXIS.
S. Ropp1, I. Bernatovska2, S. J. Paton1, W. A. Price1, M. Morris1 and D. R. Cool1.
1Pharmacology & Toxicology, Wright State University, Dayton, OH; and 2Cardiovascular Physiology, Institute of Normal and Pathological Physiology, Bratislava, Slovakia.

Pyridostigmine bromide (PB) is an acetylcholinesterase (AChE) inhibitor used as a preemptive treatment against possible exposure to organophosphate nerve agents and as a treatment for myasthenia gravis. In order to analyze the effects of PB and stress on the hypothalamic-pituitary axis (HP-axis), SELDI-TOF mass spectrometry proteomic analysis was conducted on the hypothalamus in parallel with AChE activity assays. Two models were used: PB treatment in which doses of 0.5, 1, 2, and 3 mg/kg/day were administered using Alzet minipumps implanted subcutaneously in the mice for 7 days or 7 day chronic shaker stress. The hypothalamic AChE activity in the 7-day chronic stress model was unchanged when compared to controls. PB (5 mM) was able to inhibit 98% of this activity. In contrast, a significant dose-dependent decrease in hypothalamic AChE activity (~40% at the highest dose of 3 mg/kg/day) was observed in the chronically-treated PB model. Protein analysis of the same stress and PB-treated hypothalamic homogenates by SELDI-TOF MS showed significant and similar changes in the hypothalamic peptide/protein profiles for both stressed and PB-treated mice. Ratio analysis of the protein profiles indicated a decrease in peak intensity for large (~6000) and small (~4000) peptides. However, the decrease in intensity was greater for the largest proteins (2.0-7.5 vs small, 1.3-2.0), suggesting a buildup of the smaller peptides within the cells. Analysis of the posterior pituitary also showed a similar decrease in arg-vaso-pressin peptide in both stress and PB-treated mice. These results suggest that stress
and IF both affect the peptide protein profiles in the hypothalamus by changing either the protein expression or peptide processing and secretion patterns of the cells. Supported by DoD contract No.99214005.

1087 A PROTEOMIC APPROACH TO THE CHARACTERIZATION OF ARDS.

C. D. Mobarak¹, R. P. Baughman¹ and R. E. Henderson¹, Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM and University of Cincinnati, Cincinnati, OH.

Acute Respiratory Distress Syndrome (ARDS) is a severe lung dysfunction associated with a variety of catastrophic events. The condition is characterized by bilateral infiltrates of protein-rich fluid and large numbers of neutrophils. Because ARDS can be induced by multiple factors that produce similar pulmonary pathophysiological changes, the mechanism likely involves a disturbance of basic hematogenous processes that protect and maintain normal lung permeability. Bronchoalveolar lavage fluid (BALF) contains a large number of proteins that comprise a source for testing and characterizing biochemical alterations associated with airway disease.

Proteins that are regulated, modified, secreted or excreted in the airways during disease processes can provide vital mechanistic information, as well as identify novel treatment targets. Our Lab uses proteomics techniques to identify proteins that are differentially expressed in ARDS by defining protein expression profiles (PEPs) in BALF. Human BALF samples from patients who have undergone bronchoscopy during the progression of their disease have been characterized using 2-DE gels. PDQuest software and Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry. These human PEPs are being used to guide development of an ARDS animal model. Mouse was chosen for the animal model because its genome has the most complete sequence database available for protein identification. In mice we have compared control BALF to BALF from animals with trauma induced acute lung injury. In the trauma model, BALF, the PDQuest software identified 30 protein spots that increase by at least 2-fold, 3 spots that decrease by 2-fold, and 14 spots not observed in control BALF. Glutathione S-transferase and selenium binding protein were observed in the up-regulated proteins. Correlation of human data with the mouse data will be used to validate this acute lung injury in mouse as an appropriate animal model of ARDS. Research supported by LLRI.

1088 PROTEIN EXPRESSION IN RAT TESTICULAR TISSUE FOLLOWING MULTIPLE EXPOSURE LEVELS OF JP-8 VAPORS FOR 91 DAYS.

G. B. Briggs¹, K. R. Stilll and F. Wirtzmann², Naval Health Research Center Detachment (Toxicology), WPAFB, OH, Naval Health Research Center Detachment (Toxicology), WPAFB, OH and University of Indiana, Indianapolis, IN.

JP-8 jet fuel is being universally used by the US military. The Navy is primarily concerned about vapor exposures on ships. A 91-day inhalation exposure study was conducted using BALB/c mice. The testicular tissue was harvested at necropsy and prepared for proteomic evaluation. 2D electrophoresis and peptide mass fingerprinting procedures were used to generate protein expression profiles. More than 50 proteins were identified as being specific to the testes. These proteins were excised from gels and digested with trypsin. MALDI-TOF-MS system. Increased abundance of specific proteins was observed in the low dose rats (250 mg/cubic meter). Declines in protein expression were observed in the high dose (1000 mg/cubic meter). A total of 49 changes in protein expression differences were observed in the low dose group. 24 differences in protein expression were present in the mid dose group (500 mg/cubic meter), and 26 differences were observed in the high dose group. Specific proteins that showed increased abundance in the low dose group were generally associated with cell repair, and the proteins that demonstrated decreased abundance are generally associated with cell damage. These protein expression patterns may help to understand the mechanism of cellular changes in the testes that occur prior to functional and morphological evidence of male reproductive system functional defects. Proteomics data are being used to fill current reproductive toxicity data gaps in the risk assessment of JP-8 vapor. The information is being used to help formulate appropriate exposure levels for Navy personnel in JP-8.

1089 PROTEIN PROFILING OF RAT LIVER ORGANELLES AFTER SUBACUTE PHENOBARBITAL EXPOSURE: COMPARING PROTEOMICS AND CDSN MICROARRAY DATA.

M. E. Bruno¹, J. E. Ellard¹, S. E. Javadi¹, K. T. Blanchard¹, R. E. Seel³, B. A. Wermter¹, C. H. Borchers¹, M. J. Dins¹, K. E. Tomer¹, H. Vandenbergh¹ and R. E. Merrick¹, National Center for Toxicogenomics, NIEHS, Research Triangle Park, NC, Biochemistry, UNC, Chapel Hill, NC and Toxicology, Boehringer Ingelheim, Ridgefield, CT. Sponsor: B. Merrick.

Abstract: A primary focus of toxicogenomics concerns differential gene expression produced by chemical exposure that form expression signatures that can distinguish toxicant classes and actions. Protein expression was studied in male rat liver by proteomics after subacute exposure to the metabolic enzyme inducer, phenobarbital. Proteins were fractionated into mitochondria, endoplasmic reticulum (ER), cytosol and nuclei, separated by two-dimensional (2D) gel electrophoresis, quantitated by image analysis and identified by mass spectrometry. Over 60 proteins were identified throughout all fractions including the expected induction of cytochrome P450 and glutathione transferase subunits as confirmed by western blot. Several intermediate metabolic enzymes were affected by phenobarbital in cytosolic fractions, among them carbamoyl phosphate synthetase 1, 3, 3't hydroxysteroid dehydrogenase, argininosuccinate synthetase and glyceraldehyde-3-phosphate dehydrogenase. Changes observed in nuclear samples include downregulation of LRP-p40, hnRNP A3, hnRNP A2/3 and hnRNP A1 as well as upregulation of hnRNP L. Results from Western analysis with an LPB40 specific rabbit polyclonal antibody were consistent with the 2D gel results previously observed. In addition, nine potentially new nuclear proteins were found by mass spectrometry not in public genomic databases. This study shows how proteomics might serve to establish protein signature profiles of chemical exposure in toxicogenomics studies and can also serve as an effective tool for gene discovery.

1090 IDENTIFICATION OF POTENTIAL IN VITRO ENDPOINTS FOR SKIN IRRITATION USING PROTEOMIC AND MICROARRAY ANALYSIS.

S. T. Fletcher¹, J. H. Pentselm, D. A. Bassett³, D. P. Keshel¹, M. Philipson³ and V. A. Baker¹, GE Healthcare, Uxbridge, Bedfordshire, United Kingdom and Centre for Cutaneous Research, Queen Mary, University of London, United Kingdom.

Understanding the mechanistic basis of the human skin irritation response is key to the development of relevant in vitro test systems for the predictive identification of skin irritation hazards. Recent progress in the development of proteomic and microarray technologies means that tools for the identification and investigation of important biochemical events in the processes of skin irritation are now available. This study was designed to identify proteins (and genes encoding proteins) which may be involved in the skin irritation response, following exposure of a reconstructed human skin model (EpiDerm™ (MatTek)) to the skin irritant sodium lauryl sulphate (SLS). EpiDerm™ cultures were treated in triplicate with a non-cytotoxic dose of SLS (0.1mg/ml, as determined by the MTT assay and histological examination) for 15min, 1h, 2h, 4h and 24h. Proteins were performed using 2D-gel electrophoresis (Multinph II and DALT system, APB) in combination with mass spectrometry to investigate the protein expression profile and identify proteins of interest. In addition microarray analysis was performed using DermArray (Research Genetics) cDNA arrays covering 5000 genes of relevance to skin biology. 67 proteins of potential interest were selected and identified from a range of 2D-gels. 41 of these proteins were differentially regulated in response to SLS treatment and 11 of the proteins selected were upregulated, 20 downregulated and the expression of 4 remained unchanged following exposure to SLS. Data indicated that post-translational modification occurred at an early time point (15 min) for calmodulin-like skin protein and involucrin following exposure of EpiDerm™ to SLS. Epidermal cell marker proteins demonstrated post-translational modification after 1 hour exposure to SLS. These results demonstrate the differential regulation of a number of proteins in response to a known irritant, which could represent potential new in vitro markers of skin irritation.

1091 HALOACETIC ACIDS PERTURB PROTEIN PHOSPHORYLATION IN MOUSE EMBRYOS IN VITRO.

M. R. Banton and E. S. Hunter. Reproductive Toxicology Division, USEPA, Research Triangle Park, NC. Sponsor: J. Rogers.

Halocetic Acids (HAAs) formed during the incineration process are present in drinking water. In vitro exposure of rodent oocytes to halocetic acids increased tyrosine phosphorylation including craniofacial defects. Based on the effects of HAAs in adult tissues, we tested the hypothesis that exposure to HAAs perturbs signal transduction pathways. CD-1 mouse conceptuses (3-6 somites) were exposed to HAAs at concentrations that produce 100%-dysmorphology ([1mM dichloroacetate (DCA), 350μM dibromobenzene (DBR) or 300 μM bromochloroacetate (BCA) in whole embryo culture). To determine if exposure to the HAAs perturbs protein kinase activity, embryos were radiolabelled with 33-P-phosphate in HAA-containing or control medium during 0-2, 4-6 or 22-24 hour exposure. 2D gel electrophoresis was then performed and autoradiographs compared using Phoretix 2D Software (Nonlinear Dynamics). Both time and chemical changes in protein phosphorylation were observed. To further evaluate protein phosphorylation, Western analysis on 1D gels was conducted using a phospho-tyrosine antibody (PY99: Santa Cruz Biotechnology). Two major phosphorysine containing proteins (122 and 183kDa) were found to be affected by HAA exposure. No changes were observed as early as 2 hours after treatment but an increase in the 122kDa protein was found 6 hours following DCA exposure. By 24 hour after culture both phospho-
proteins were increased in all three HAA treated groups with DCA producing the greatest effect. These changes indicate that the HAA perturb signal transduction by altering the phosphorylation state of phospho-cytosine proteins. Since phospho-tyrosine kinase inhibitors also perturb phagocytosis, HAA-induced alteration of signal transduction may be responsible for altered differentiation and development. This abstract does not present EPA policy.

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LOCALIZATION OF IKB AND IKK SUBUNITS IN THE SMOOTH ENDOPASIMAL RETICULUM: ACUTE EFFECTS OF TCD2 IN MALE AND FEMALE RAT LIVER.


Effects of acute 2, 3, 7, 8-tetrachlorodibenzofuran (TCDD) exposure upon protein expression in rat liver endoplasmic reticulum (ER) were determined by two-dimensional gel separation and mass spectrometry. Liver ER was prepared as microsomes from male and female Sprague-Dawley rats exposed for 72 hr to TCDD. TCDD induced cytochrome P450 enzymes, GST subunits, Hsp72 and Grp78. Mass spectrometry also revealed the presence of increased levels of IKK kinase family (IKK-b) after dioxin treatment in males and females. This finding led us to search for other NF-kB binding proteins or kinases in ER and possible alterations by TCDD. Western blot for IKK family members in ER showed that IKK-b and IKK-epsilon were highly expressed in male while IKK-beta and IKK epsilon were barely detectable. TCDD induced hyperphosphorylated forms of IKK-b in both genders, but only the 85kd form of IKK-gamma was present in females while males expressed an additional IKK-gamma immunoreactive form at 115 KD. Although IKK-beta was in ER of both genders, IKK-gamma was the most abundant IKK subunit and IKK-gamma was not detectable. The effect of gender and TCDD treatment upon IKK and IKK family member expression was also examined in cytosol for critical comparison to the ER. In cytosol, all IKK subunits were expressed with substantial effect by TCDD. IKK-alpha, IKK-beta, and IKK-gamma were observed in cytosol only to females (not male) rats but were not increased by TCDD as was the case in ER from females. Although NF-kB p65 was readily found in male and female cytosol, it was not detectable in ER. IKK-beta kinase activity, measured after immunoprecipitation from cytosol and solubilized ER, was unaffected by gender and treatment. These studies demonstrate a strength of proteins in showing a unique distribution of NF-kB binding proteins and their kinases in liver ER as affected by gender and TCDD treatment.

1093

T-2 AND HT-2 TOXIN INDUCED APOPTOSIS IN HL-60 HUMAN PROMYELOCYTIC LEUKAEMIA CELLS.


T-2 and HT-2 toxins belong to a group of mycotoxins, trichothecenes, which are widely encountered as natural contaminants. Exposure to such toxins may cause severe damage to the gastrointestinal mucosa and the immune system. In the present study the human promyeocytic cell line HL-60, was used to characterize the apoptotic effects of T-2 and its metabolite HT-2. Apoptotic cells were identified microbiologically by chromatin condensation and nuclear fragmentation, by flow cytometry analysis and by DNA ladder. Apoptosis induced in the HL-60 cells following exposure to T-2 and HT-2 for 24 hours was concentration-dependent starting at 3.1 and 6.25 ng/ml, respectively. Little cytotoxicity (membrane damage) was observed even after exposure to concentrations (25-50 ng/ml) that induced 60-100% of the cells to undergo apoptosis. Following exposure to 6.5 ng/ml an increased amount of apoptotic cells could be observed already after 4.5 hours. Whereas the absolute effects of toxin-induced apoptosis in the HL-60 cells seemed to vary somewhat during culturing and between different batches of cells, T-2 was more potent than HT-2. The apoptotic process could be almost completely blocked by the addition of s-VAD FMK. In contrast, no or only minor effects were observed by the addition of DEVDCHO, IETD FMK and DEVD FMK. As judged by Western blotting, no or only minor changes in box and bel-2 levels were observed, whereas PARP was totally degraded already 3 hours after toxin addition. Furthermore, the level of several proapoptotic (-3, -7, -8, -9, but not -2) were down regulated. Zn + and BAFA-AM blocked induction of apoptosis as judged by microscopic analysis and flowcytometry, as well as DNA fragmentation. The results suggest that T-2 and HT-2 both cause an increase in the level of Ca + thereby initiating a signal that activates several caspases resulting in chromosomal condensation, nuclear fragmentation, activation of DNA endonucleases and DNA ladder.

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ALTERATIONS IN EXPRESSION OF CYTOKINE NETWORK AND APOTOSIS SIGNALING GENES IN MOUSE LIVER AFTER SUBCUTaneous FUMONISIN B1 EXPOSURE.

N. Bhandari and R. P. Sharma. Physiology and Pharmacology, University of Georgia, Athens, GA.

Fumonisins B1 (FB1) is a naturally occurring mycotoxin produced primarily by Fusarium verticillioides and related fungi, which are common contaminants of corn throughout the world. FB1 is a carcinogenic and causative agent of several lethal animal diseases. Liver is the primary target organ in mice. Our previous studies showed altered expression of cytokines in mouse liver after FB1 treatment. To further investigate the genes involved in the cytokine network and apoptosis signaling, male and female BALB/c mice (5/group) were injected subcutaneously with either saline or 2.25 mg/kg/day of FB1, for 5 days. FB1, treatment caused increased expression of tumor necrosis factor alpha (TNF alpha), interleukin (IL)-1alpha, IL-1beta, IL-6, IL-10, IL-12 p40, IL-18 and interferon gamma (IFN gamma) in male liver, with a similar increase in females except that IL-1beta and IL-18 were unaltered. Control female mice injected with saline in their right hind footpads did not show any alterations. Saline was administered to both male and female mice in the same manner. FB1 and IFN gamma as compared to males, expression of TNF receptor 55 and TNF receptor associated death domain (TRADD) was increased, with no changes in IFN signaling molecules, Fas, Fas ligand, Fas associated death domain (FADD) and Fas-associated protein factor (FAP). Expression of c-Myc, B-Myc, Max and Mad oncoprotein transcription factors and apoptotic genes in early (IL-1beta, IL-6, IL-12 p40, IL-18, IL-10, IL-12 p70 and IFN gamma) as well as increased after FB1 treatment, FB1, treatment caused an activation of cytokine network in liver along with TNFalpha signaling pathways. FB1, induced expression of TNFalpha, IL-1alpha, IL-1beta, IL-6 and IFNgamma could play a role in the observed liver toxicity, whereas increased expression of IL-1alpha and oncoerys could be responsible for the cancer promoting properties of FB1.

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IMMUNOTOXIC EFFECTS OF ENDOSULFAN AND PERMETHRIN IN THYMCYTOPE APOPTOSIS.

J. J. Keenan, H. P. Mira, E. Adzham, and H. Cold饶lberg. 1Biomedical Sciences & Pathobiology, Virginia Tech, Blacksburg, VA and 2ORD, USEPA, Cincinnati, OH.

Altered immune responses have been observed following occupational, inadvertent, or therapeutic exposure to xenobiotics. Many pesticides are known to cause immunotoxicity. Exposure to mixtures of pesticides, either concurrently or sequentially, may result in potentiating this effect mainly because one can effect the metabolism of the other. The objective of this study was to determine the effect the interactions between their mixtures and their individual pesticides in mice thymocytes in vitro. Permethrin is a broad-spectrum synthetic pyrethroid, is a widely used insecticide in agriculture and public health. Endosulfan is a highly toxic chlorinated hydrocarbon insecticide used worldwide. We examined the immunotoxic potential of these pesticides using a flow cytometric technique in combination with 7-Amino Actinomycin D (7-AAD) to study the early apoptotic cell death, respectively. Endosulfan at 25, 50 and 100 μM was found to cause 5.49, 25.21, and 66.1% early apoptotic cell death, respectively. For the mixture study, concentrations of 100 μM permethrin and 50 μM endosulfan was selected and found to cause 27.09% apoptosis. Thus, these pesticides in mixture have an additive immunotoxic effect. No significant later apoptotic cells were found in these concentrations for either pesticide when exposed for 12 hours. DNA ladder assay confirmed the presence of DNA fragments. The results of this study suggest that the mixtures of endosulfan and permethrin have additive immunotoxic effects on C57BL/6 mice thymocytes.

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OXIDIZED PHOSPHATIDYLSERINE STIMULATES PHAGOCYTOSIS OF APOTOTIC CELLS.

C. A. Smith1, S. X. Liu1, F. B. Serrink1, V. A. Turin2 and V. E. Kagan3.

1Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, PA and 2Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Science, St. Petersburg, Russian Federation.

A critical event during programmed cell death or apoptosis is the acquisition of plasma membrane changes that allows phagocytes to recognize and engulf cells before they rupture. Externalization of phosphatidylserine (PS) has been considered a hallmark of apoptosis. Oxidative stress is an intrinsic part of the apoptotic program with an unknown specific function. We have previously found that apoptosis induced by oxidants and non-oxidants such as phorbol myristate acetate (PMA) and anti-Fas, was accompanied with PS oxidation and externalization in apoptotic cells. We hypothesized that PS oxidation plays important role in recognition and phagocytosis of apoptotic cells by macrophages. To test this hypothesis, 1-palmitoyl

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(C16:0)-2-arachidonyl) (20:4) PS was oxidized by incubation with 2, 2′-azo-bis(2-aminopropane) (H2O2) and incorporated into plasma membrane of HL-60 cells. By comparing phagocytosis of cells with PS oxidation (PSox) by macrophage J774.A1 cell line, we found that PSox strongly enhanced engulfment of cells by macrophages. Another phospholipid, phosphatidylincholine (PC), when oxidized to the same extent as PS, had no effect on phagocytosis. Liposomes containing PSox inhibited phagocytosis of apoptotic HL-60 cells induced by tumor necrosis factor (TNF) by macrophages more potently than those containing PS. Based on our experiments with different receptor-specific antibodies, uptake of cells with incorporated PSox was blocked 50% by mAb against both PS-R and Ox-LDL, and 40% by mAb against both CD36 and RGDS. This implies that CD36 and the lectin receptor might be responsible for recognition of PSox while the PS receptor and the scavenger receptor, CD36, might be involved in recognition of PS. These results suggest that PS oxidation is likely to be a new signaling pathway for clearance of apoptotic cells by macrophages.

1097 VITAMIN E AND RECOGNITION/CLEARANCE OF APOPTOTIC JURKAT CELLS BY MACROPHAGES.


Phagocytosis of apoptotic cells is important for effective clearance of dead cells without inflammatory reaction. PS externalization, a hallmark of apoptosis, is required for recognition of apoptotic cells by macrophages. Specific PS peroxidation has been documented during apoptosis and suggested to participate in PS-dependent signaling pathways. This implies that antioxidants may affect recognition of apoptotic cells by macrophages via inhibition of PS peroxidation. Apoptosis was induced by anti-Fas mAb in Jurkat cells after 48 h starvation with different concentrations of D,L-α-tocopherol (Toc). Intracellular vitamin E (VE) concentrations were measured by HPLC. PS oxidation was assessed by HPLC after metabolic labeling of cells with oxidation-sensitive cis-punicic acid. Apoptosis was assessed by nuclear morphology using Hoechst 33342 staining. For phagocytosis, we used J774.A1 murine macrophage cell line. Percentage of apoptotic cells was 70 ± 1, 11 ± 3, 32 ± 1, 28 ± 3, 35 ± 3 in the control without any treatment, VE without anti-Fas mAb, anti-Fas mAb without VE, and 0.25, 2.5, 50 μM VE plus anti-Fas mAb, respectively. Percentage of phagocytosis-positive macrophages was 5 ± 1, 5 ± 2, 25 ± 3, 34 ± 4, 27 ± 8, 29 ± 4 in control without any treatment, VE without anti-Fas mAb, anti-Fas mAb without VE, and 0.25, 2.5, 50 μM VE plus anti-Fas mAb, respectively. These results show that VE treatment had no significant effect on phagocytosis of apoptotic Jurkat cells. Although PS externalization may be one of the final common pathways of apoptotic program that makes the cells recognizable by macrophages, peroxidation of PS can play a role as an additional signal for one or more macrophage receptors. It is important to determine whether other lipid- and water-soluble antioxidants may affect phagocytosis of apoptotic cells via inhibition of PS peroxidation.

1098 HOW MUCH PHOSPHATIDYL SERINE (PS) IS ENOUGH FOR MACROPHAGES TO RECOGNIZE TARGET APOPTOTIC CELLS?


PS usually confined to the internal leaflet of plasma membrane (PM) is externalized on the cell surface during early apoptosis. Externalized PS (PSe) is required for effective phagocytosis and clearance of apoptotic cells by macrophages (MF). Others & we found in normal cells PS asymmetry is not absolute suggesting the amount of PS on cell surface is important for MF recognition of dead in live cells. Therefore, we developed a new sensitive method to quantify the amount of PSe. This method is based on the binding of annexin V conjugated with iron beads to PS. Because the beads have a strong iron EPR signal cells containing PS can be labeled with annexin V-Fe (AV-Fe) and assayed by EPR. We prepared Jurkat cells with known amounts of added PS on their surface by fusing them with PS-containing liposomes. EPR signal magnitude from AV-Fe labeled cells linearly depended on the amount of PSe. AV-Fe labeling was specific due to annexin-PSe interaction as confirmed by low EPR signal magnitude from cells labeled with beads alone. We found that normal cells on average contain ~0.04 nmol of endogenous PSe/10^6 cells. To determine the level of PS sufficient for triggering by hypoxiare, we prepared cells with different amounts of PSe. Phagocytosis percent as determined by fluorescence microscopy depended non-linearly on the level of PSe. In contrast to linear elevation of PSe, phagocytosis had a pronounced lag-phase at low amounts of PSe increased markedly, when PSe exceeded 0.2 nmol/10^6 cells. EPR signal from cells in which apoptosis was induced by camptothecine revealed that at least 1 and 4 nmol PSe/10^6 cells were externalized after 1 and 4 h. Thus, MF have a sensitivity threshold for the amount of PS on the surface of target cells. Normal cells contain very low levels of PSe that dramatically increased during apoptosis providing reliable recognition of apoptotic cells by MF.

1099 ATTENUATION OF CD95-INDUCED T CELL APOPTOSIS BY INORGANIC MERCURY: COMPARISON BETWEEN THE EFFECTS OF MERCURY AND OTHER GROUP II METALS.

G. McCollum, A. J. Rosenzweig, and M. J. McCabe. Department of Environmental Medicine, University of Rochester, Rochester, NY, and Department of Biological Sciences, Wayne State University, Detroit, MI.

Mercury (Hg), a metal implicated in systemic autoimmune disease, selectively dysregulates CD95-mediated apoptosis. Such dysregulation may be one mechanism that contributes to the accumulation of autoimmune lymphocytes. We have found that submicromolar Hg concentrations impair CD95 induced apoptosis in Type-1 (HUT-78) and Type-II (Jurkat) T cell lines as well as in normal human activated CD4+ T cells. Hg blocked the CD95-induced activation of caspase-8, caspase-3 and -7, as well as the association between CD95 and the signaling adaptor, Fas-associated protein with death domain. Multimerization of CD95 on the cell surface was not affected by Hg. Thus, the Hg sensitive step within the CD95 death pathway localizes to the death inducing signaling complex (DISC). Disruption of DISC formation and the downstream activation of caspases may be a mechanism whereby Hg contributes to autoactivity. Although no clear linkage between other Group II metals (i.e., cadmium, zinc) and autoactivity have been established, these metals have been shown to inhibit apoptosis in various cells. Thus, we compared the effects of Cd and Zn with Hg on CD95-induced apoptosis. Using Jurkat cells, the influences of Cd and Zn on CD95-mediated caspase-3 activation were investigated. Caspase-3 is an important effector of apoptosis in many autoimmune reactions and is convergent at its activation. Caspase-3 enzyme activity was assessed in CD95-sensitized Jurkat cells exposed to Cd (0.055 M) or Zn (5.50 M). A dose-dependent inhibition of caspase-3 activity was noted for both Cd and Zn; however, unlike Hg we suspect that this caspase-3 inhibition is not selective for the CD95 pathway. Cd and Zn likely inhibit caspase-3 directly. As such, CD95-mediated apoptosis is not selectively inhibited by Cd and Zn inhibition, which may explain why there is limited evidence linking these metals with autoimmune disease. Supported by R21ES10351 and T32ES07026.

1100 BIOLUMINESCENT DETECTION OF P53 IN VITRO AND IN VIVO IN RESPONSE TO DNA DAMAGING AGENTS.


We have designed tumor cell-based models with the potential to detect p53 activity following exposure to genotoxic agents. P53, a key regulator of apoptotic activity in response to DNA damage, is frequently mutated, absent, or modulated in tumor cells. For this study, a luciferase reporter vector that contained p33-response elements upstream of a minimal TK promoter and the luciferase structural gene (p53RE-luc) was used to detect activation of p53 pathways by bioluminescence. Two human lung carcinoma cell lines, A549 cells (p53wt) and H1299 cells (p53-/-), were transfected with p53RE-luc, treated with doxorubicin or cisplatin. Luciferase activity was measured 24 and 48 hours post-treatment. In A549 cells, doxorubicin (0.01 to 0.30 μM) and cisplatin (0.30 μM to 30 μM) produced dose-related increases in luciferase activity when compared to vehicle-treated cells. As expected, the activity was diminished in treated p53-/- cells since these cells do not express p53. These results suggest that the observed response of A549 cells to genotoxic agents was mediated by p53. When H1299 and A549 cells were cotransfected with a p53-expression vector and p53RE-luc, p53 expression was dramatically induced in H1299 cells but not in A549 cells. These findings indicate that A549 cells, which are p53 competent, may modulate p53 activity by post-translational modifications. Western blot analysis showed increased phosphorylation of p53 at Ser15 following doxorubicin treatment. This modification has been associated with increased p53 activity. Athymic mice implanted with these bioluminescent reporter cell lines showed increased p53 activity within 6 hr after doxorubicin treatment. We have subsequently obtained in vivo data. These findings suggest that the p53RE-luc reporter may provide a rapid, in vivo assay that can be used to detect the activity of DNA damaging agents.

1101 SENSITIZATION OF DRUG-RESISTANT B-LYMPHOMA CELLS TO AISENITE-INDUCED APOPTOSIS BY PHARMACOLOGICAL MANIPULATION OF MAP KINASE PATHWAYS.

D. E. Muscarella, Microbiology and Immunology, Cornell University, Ithaca, NY. Sponsor: S. Bloom.

Trivalent arsenic, or arsenite, is a potent inducer of mitogen-activated protein kinase (MAP) pathways, stress proteins, and apoptosis, and is clinically useful in the treatment of some hematopoietic malignancies. However, the signaling pathways...
that regulate the induction of apoptosis by arsenite in hematopoetic cells are only partially known. I studied the activation of MAP kinases by sodium arsenite in a panel of cell lines derived from human Burkitt's lymphoma that show marked differences in their susceptibility to apoptosis induction, and investigated the hypothesis that treatments that effectively modulate MAP kinase activity can prevent apoptosis induction in resistant cells. I found that the apoptosis-sensitive ST486 cell line differed from the apoptosis-resistant EW36 cell line, which over-expresses the Bcl-2 protein, in terms of the requirement for activation of the c-jun N-terminal kinase (JNK) pathway for apoptosis induction. Specifically, the sensitive cell line, ST486, underwent extensive arsenite-induced apoptosis at concentrations below 20 micromolar, while the resistant EW36 cell line was relatively resistant. I then investigated the possibility that arsenite-induced apoptosis in the sensitive cell line was due to JNK activation, which occurs only at higher concentrations of arsenite exposure, was required for apoptosis induction in the EW36 cell line. Furthermore, the EW36 cells were substantially sensitized to arsenite-induced apoptosis by exposure to p38 inhibitor, a non-lymphoid hyperthermia. Moreover, pretreatment of EW36 cells with a pharmacological JNK inhibitor of p38 MAP kinase also potentiated the arsenite-induced apoptosis, and acted synergistically with hyperthermia to further sensitize these cells. Significantly, the experiments revealed that sensitization of EW36 cells is mediated by activation of the JNK pathway and that pharmacological inhibition of the p38 pathway acts synergistically with hyperthermia to overcome Bcl-2 mediated resistance to apoptosis by prolonging a transient period of JNK phosphorylation that occurs immediately after heat shock treatment. (Supported by NIH grant ES09185)

1104 OXIDATION AND EXTERNALIZATION OF PHOSPHATIDYLSERINE ON ACTIVATED NEUTROPHIL-LIKE CELLS. ROLE IN APOPTOSIS AND PHAGOCYTIC CLEARANCE.

A. Arroyo*, M. Modrianska, V. P. Seresnak, R. J. Belly, T. Matsuura, V. A. Turijn, Y. Y. Turina and V. V. Kagan

Environ Health & Occupational Health, University of Pittsburgh, Pittsburgh, PA; Medical Chemistry & Biochemistry, Palacký University, Olomouc, Czech Republic and Cell Biology, Physiology & Immunology, University of Córdoba, Córdoba, Spain.

To prevent calcium release of reactive oxygen species (ROS), macrophages remove excess activated neutrophils from an inflammatory site in a regulated way, through processes that ensure the efficient resolution of inflammation, and maintenance of tissue repair and homeostasis. The focus of our current work is on the role of macrophages in the resolution of neutrophilic inflammation in the lung.

To advance our understanding of neutrophilic inflammation, we used a mouse model of pulmonary inflammation induced by bleomycin, a clinically relevant model of epithelial injury and fibrosis. We observed that neutrophils in the lung periphery were rapidly phagocytosed by alveolar macrophages, suggesting that activated neutrophils are efficiently cleared by alveolar macrophages.

We next investigated the role of phosphatidylserine (PS), a membrane lipid that is exposed on the surface of dying cells, in the clearance of neutrophils by alveolar macrophages. We found that neutrophils expressing PS were efficiently cleared by alveolar macrophages, whereas neutrophils lacking PS were not.

These findings suggest that PS expression on the surface of neutrophils is important for their clearance by alveolar macrophages, and may provide new insights into the mechanisms of phagocytosis and resolution of neutrophilic inflammation in the lung.

1105 CYCLOPENTANYL POLYCYCLIC AROMATIC HYDROCARBONS INDUCE CYTOCHROME P450 1A1, ACCUMULATION OF CELLS IN S PHASE AND APOPTOSIS IN MOUSE HEPATOMA (HEPA 1C1C7) CELLS.


Cyclopentane polycyclic aromatic hydrocarbons (CP-PAH) are formed by incomplete combustion of organic material. The CP-PAH cyclopentane, diphenyl (CYP) has been detected in diesel and automobile exhaust at levels as high as 10000 ppb. It is a well-known PAH benzo[pyrene (B[p]P). CYP has been found to be a potent carcinogen in both bacterial and mammalian cells, and to induce tumors in various sites in the body.

To investigate the effects of CP-PAHs on Hepa 1c17 cells, we cultured Hepa 1c17 cells in the presence of various concentrations of CP-PAHs. We observed that CP-PAHs induced cell death in Hepa 1c17 cells in a dose-dependent manner. Additionally, we observed that CP-PAHs induced cell death in Hepa 1c17 cells in a time-dependent manner.

To determine the mechanism of cell death, we performed apoptosis and survival assays. We observed that CP-PAHs induced apoptosis in Hepa 1c17 cells, as evidenced by an increase in the number of annexin V-positive cells. Furthermore, we observed a decrease in the number of viable cells.

To determine the role of CP-PAHs in cell cycle progression, we performed flow cytometry analysis. We observed that CP-PAHs induced a decrease in the number of cells in the G0/G1 phase, and an increase in the number of cells in the S phase.

In conclusion, our findings suggest that CP-PAHs induce cell death in Hepa 1c17 cells through apoptosis and cell cycle progression. These findings provide new insights into the mechanisms of CP-PAH-induced cell death in Hepa 1c17 cells.
P38-MITOGEN-ACTIVATED-PROTEIN-KINASE-MEDIATED BAX TRANSLATION INITIATES CYTOCHROME C RELEASE IN CYANIDE-INDUCED APOPTOSIS.

Y. Zhao, J. L. Baxevanis, and G. E. Iovov. Medicinal Chemistry and Molecular Pharmacology, Purdue University West Lafayette, IN.

Cytosolic cytochrome c release from mitochondria is a critical event in cyanide-induced apoptosis. To determine how cytochrome c translation-initiated cytochrome c release, we investigated endogenous Bax translocation and apoptosis in primary cultured rat cortical neurons. Under normal conditions, Bax resided mainly in cytoplasm. After 30 min of cyanide treatment for 1 hr, Bax translocated specifically to the mitochondria as shown by immunocytochemical staining and western blotting analysis. Bax translocation preceded cytochrome c release from the mitochondria, which was initiated 3 hr after cyanide treatment. To determine that the translocation of Bax to mitochondria initiated cytochrome c release, double immunofluorescence labeling for both Bax and cytochrome c was used. This study showed that cytochrome c was released only in cells harboring Bax in mitochondria. In cells where Bax translocation did not occur, cytochrome c was still associated with mitochondria. P38 mitogen-activated protein (MAP) kinase was involved in regulating Bax translocation in cyanide-induced apoptosis. This kinase was activated 30 min after cyanide and its phosphorylation began to decrease 3 hr later. SB203580, a specific p38 MAP kinase inhibitor, blocked translocation of Bax to mitochondria. Inhibition of p38 MAP kinase by SB203580 blocked all downstream effects of Bax translocation including cytochrome c release, caspase activation and DNA fragmentation. These results demonstrated that Bax translocation was critical for cytochrome c induced cytochrome c release and p38 MAP kinase was involved in regulating Bax translocation from the cytosol to mitochondria. (Supported by NIH grant 54140)

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APOTOTIC EFFECT OF LINOILEIC ACID ON 15LO OVEREXPRESSION ENDOTHelial CELLS.


The development of atherosclerosis is postulated to occur via endothelium alterations caused by unsaturated fatty acids and inflammatory cytokines. To test the hypothesis that 15-lipoxygenase (15-LO), a non-enzyme-containing enzyme, causes oxidant injury and apoptosis in endothelial cells the following experiment was conducted. ECV-304 (parental line) and ECV-LO cells (ECV-304 cells stably transfected with human 15-LO) were treated for up to 4 hr with 0, 50, 100, 200 µM linoleic acid (LA) and/or 7.5 µM NDGA, a non-specific inhibitor of lipoxygenase. Apoptosis was monitored by measuring changes in cellular status such as oxidation stress, intracellular calcium homeostasis, F-actin content, nuclear morphology, mitochondrial potential and c-Jun N-terminal kinase (JNK) activity. A 2-fold increase in oxidative stress occurred in ECV-LO compared to ECV-304 cells within 30 min of 200 µM LA. Nuclear fragmentation, decreased mitochondrial potential, and a significant decrease in F-actin content were seen within 15-60 min of 100 µM LA treatment in ECV-LO cells. Similarly, significant decreases in F-actin content and mitochondrial potential were seen in parental cells compared to control. However, these cells also had significant nuclear condensation within 30-60 min of treatment. Pretreatment of ECV-LO cells with NDGA inhibited nuclear fragmentation caused by 100 µM LA, but had no effect on decreases in mitochondrial potential or F-actin content. No effect on JNK activity was noted. Significant increases in intracellular calcium occurred in ECV-LO cells within 15 min of LA treatment. These results suggest that LA produces DNA fragmentation and oxidative stress in the endothelial as early as 15 min posttreatment. Mobilization of intracellular calcium may contribute to the apoptotic events observed in these cells.

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DMBA-INDUCED PRE-B CELL APOPTOSIS: INTRACELLULAR SIGNALING PATHWAYS.


The purpose of this study was to investigate intracellular signaling pathways of PAH-mediated, pre-B cell apoptosis and to compare these pathways to those activated in immortal B cells. Apoptosis of immortal WEHI-231 B cells induced by 10 ng/ml of the pyrrolizidine alkaloid, dexamethasone (Dex), which also induces B cell apoptosis, has been used to define apoptosis mechanisms in these cells. In both cases, DNA fragmentation preceded by an increase in expression of p21 

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1111 ENANTIOSELECTIVE METABOLISM AND INHIBITION OF GLUTATHIONE TRANSFERASE-ZETA BY BROMOCHLOROACETIC ACID.


Bromochloroacetic acid (BCA) is a chiral di-halocarboxylic acid that may arise as a by-product of dioxin contamination in defoliation. Chlorinated and brominated di-halogenated acids are metabolized by glutathione transferase zetas (GSTZ1-1) to either form glycinate or cause suicide inactivation of the enzyme. To better understand the molecular events involved in (-), (+)-BCA metabolism, we expressed and purified recombinant mouse GSTZ1-1 and examined the metabolism and suicide substrate qualities of the separate enantiomers of BCA. We also studied the effect of in vivo exposure to (-) and (+)-BCA on the GSTZ1-1 activity in hepatic cytosol from male F344 rats. Exposure to (-) and (+)-BCA concentrations as low as 1.4 mg/l were found to decrease GSTZ1-1 activity in liver cytosol. These results indicate that BCA is a potent mechanism-based inhibitor of GSTZ1-1 and its metabolism to glycinate is controlled by an additional GST enzyme at yet uncharacterized.

Supported by EPA STAR grant R825954.

1112 ACYLAMIDE: METABOLISM AND HEMOGLOBIN ADDUCTS FOLLOWING INTRAPERITONEAL, DERMAL, OR INHALATION EXPOSURE.

S. C. Sannier, C. C. Williams, R. Snyder, W. Kuril and T. R. Fennell. CITT Center for Health Research, Research Triangle Park, NC.

Occupational exposure to acrylamide (AM) via dermal contact or inhalation may occur during use as an intermediate in the production of polymers, or during the use of AM-containing coatings as insulating materials. In this study, AM-derived urinary metabolites and hemoglobin adducts were measured in rats and mice following inhalation exposure and in rats following intraperitoneal (ip) and dermal administration. No mortality occurred from a 6-hr exposure to 3.0 ppm AM-vapor. Rats exposed for 6 hr to 3 ppm (C)AM despair from an upper limit of (90) ppm (C)AM vapors had a lower uptake (90) ppm (C)AM vapors. AM-derived (C)AC was detected in 24-hr urine and feces of 42% of recovered (C)AC in rats. 51% of mice) or recovered in tissues (36% rats, 46% mice). Following a dermal application of 162 mg/kg (C)AC for 6 hr, 22% of the applied dose was absorbed and distributed to the excreta (44%) or tissues (53%) by 24 hr. Following a ip administration of (C)ACAM, 62% of the dose was recovered in 24-hr urine. In rats, metabolites derived from AM conjugation with glutathione (AM-GSH) accounted for 69% (%), 62% (dermal) and 64% (inhalation) of the urinary metabolites. In mice, the GSH pathway accounted for approximately 27% of the urinary metabolites. Remaining metabolites were derived from oxidation of AM to glycinate (GA), Hemoglobin adducts of (C)AC [N-(C-aminoethyl)cysteine] (C)AC and (C)GA were analyzed by liquid chromatography mass spectrometry. AAVAL in rats was 12900 ± 652 (ip), 6389 ± 2979 (dermal), and 1207 ± 127 (mg/m²/hr) in inhalation, while GAAV in rats was 9031 ± 646 (ip), 10010 ± 3149 (dermal), and 3339 ± 206 (mg/m²/hr) in inhalation. In mice, AAVAL was 1431 ± 509, and GAAV was (8453 ± 64) mg/m²/hr (inhalation). GAAV:VAVL ratios were 0.7 (ip), 1.56 (dermal rat), 1.3 (inhalation rat), and 1.5 (inhalation rat). This mouse study demonstrated marked species and route differences in the metabolism and internal dose (HB-adducts) of AM and GA. (Supported by the Peltz-Electrode Products Group).

1113 BIOTRANSFORMATION OF 1,1,1,3,3-PENTAFLUOROPROPANE.

T. Bayer, W. Dekanter, A. Ambros, G. E. Rusch and M. W. Anders. Department of Toxicology, University of Würzburg, Würzburg, Germany, Honeywell, Morris, NJ and Department of Pharmacology and Physiology University of Rochester, Rochester, NY.

1, 1, 1, 3, 3-Pentfluoropropane (HFC-245fa) is developed as an alternative to replace chlorofluorocarbons. HFC-245fa has only low toxicity, the only toxic effect seen in rats after inhalation exposure to HFC-245fa in concentrations of up to 50 000 ppm for 90 days was an increased incidence of diffuse myocarditis. To elucidate the role of biotransformation in HFC-245fa toxicity, we studied its bioactivation in rats after inhalation and in rat and human liver microsomes. Rats (n = 5) were exposed to 50 000, 10 000, and 2000 ppm HFC-245fa for 6 hr, urine was collected for 72 hr and metabolites exceeded were identified by 19F-NMR and quantified by GC/MS. Trifluoroacetic acid and inorganic fluoride were identified as major urinary metabolites of HFC-245fa. 11H, 11, 11-trifluoropropan-2-one was also a major metabolite. After inhalation exposure to 50 000 ppm HFC-245fa, a total of 9.9 ± 4.11 mg of trifluoroacetic acid were recovered in urine in men and 3.0 ± 0.41 mg in females; trifluoroacetic acid in urine amounted to 79.3 ± 14.3 mg in male and 9.1 ± 3.3 mg in female rats. Extent of HFC-245fa biotransformation after inhalation of 50 000 ppm and 2 000 ppm was linearly dependent on exposure concentrations. In liver microsomes, HFC-245fa was transformed following the hydroxylation scheme 04 dependent reaction to trifluoroacetic acid and trifluoroacetic acid. Rates of formation of trifluoroacetic acid were 99.2 ± 20.5 pmol/mg x min and of trifluoroacetic acid were 17.5 ± 4.0 pmol/mg x min in rat liver microsomes. In human liver microsomes, rates of formation of trifluoroacetic acid ranged from 0.7 to 7.6 pmol/mg x min and rates of formation of trifluoroacetic acid ranged from 0.7 to 7.6 pmol/mg x min and rates of formation of triglyceride were 59.3 ± 15.8 pmol/mg x min.

The obtained results suggest that HFC-245fa is metabolized at very low rates in vitro and in vivo. The toxic effects of HFC-245fa may be due to the formation of the minor metabolite trifluoroacetic acid, which is highly toxic in rats.

1114 TOXICOKINETICS OF BIPHENYL A IN HUMAN SUBJECTS.

W. Dekanter, E. Lederer, N. Wolf, T. Colnot and V. Wölkel. Department of Toxicology, University of Würzburg, Würzburg, Germany.

Biphenyl A is a widely used chemical and a weak estrogen in vitro and in rats in vivo. In rats, biphenyl A is only slowly eliminated after oral administration due to enterohepatic circulation of metabolites. To characterize toxicokinetics of biphenyl A in humans, 3 male and 3 female human subjects were orally given 5 mg d5-biphenyl A. d16-Biphenyl A was used due to background concentrations of d16-biphenyl A interfering with quantitation. Urine and blood concentrations of d16-biphenyl A and its glucuronide were determined by GCMS and LC/MS MS at different time points. The study was performed with informed consent by the human subjects and approved by the local institutional review board. Free d16-biphenyl A could not be detected in blood and urine samples after d16-biphenyl A administration; the administered dose was completely recovered in urine as d16-biphenyl A glucuronide within 24 h. Biphenyl A glucuronide was cleared by first-order kinetics from blood and urine within half-lives of 6 h, no gender differences were observed in maximum blood concentrations and in rates of excretion. The obtained data show that d16-biphenyl A is completely absorbed from the gastrointestinal tract in humans after giving low doses and subject to a complete first-pass metabolism to give d16-biphenyl A glucuronide which is rapidly excreted. The species differences in d16-biphenyl A excretion kinetics between humans and rats are most likely due to the different threshold for biliary elimination. Due to rapid biotransformation to the glucuronide, bioavailability of the weakly estrogenic biphenyl A is very low and accumulation in the human body is not expected due to rapid excretion. This work was supported by Umweltbundesamt, Berlin, Germany.

1115 EXPRESSION AND ACTIVITY OF A NOVEL MUTATION IN THE HUMAN FLAVIN-CONTAINING MONOXYGENASE 3 GENE.

B. Farnes, J. Zhang, J. Cashman and D. Schijf. Environmental Sciences, University of California, Riverside, Riverside, CA and Human Biomolecular Research Institute, San Diego, CA.

Flavin-containing monooxygenases are microsomal drug- and pesticide-metabolizing enzymes implicated in the NADPH-dependent oxidation of a variety of soft nucleophiles. The FMO family comprise 5 isoforms, each demonstrating ontologic, species- and tissue-specific regulation. The major human isoform, liver FMO3, catalyzes the N-oxidation of trimethylen. Activity of FMO3 is deficient in patients diagnosed with the autosomal recessive human disorder trimethylenuria. Trimethylenuria patients excrete relatively large amounts of trimethylenuria and results in a fish-like odor of the urine, sweat and breath. Hypercyclic stresses following consumption of tyramine-containing foods as well as clinical depression have been observed in patients suffering from trimethylenuria. As part of a larger effort to elucidate the frequency and polymorphism in the FMO family of genes in African-Americans, a novel mutation in FMO3 was found. One individual expressing the mutation demonstrated phenotypic signs of trimethylenuria, but another did not. The mutation is a C117T transition at codon 132, resulting in a change from aspartic acid to histidine. The 132 codon is in a region that is well
conserved within the human FMO family. We introduced the mutation into wild-type FMO3 using site-directed mutagenesis and expressed both wild-type and mutated FMO3 as maltose-binding fusion proteins using the pMAL-c2 (pmbpl) bacterial J.109 strain. The proteins were purified on an amylose-resin column and the activity of the enzyme was investigated with regards to trimethyloxamine, 5-DPT (10-(N,N-Dimethylamino)ethyl-2-(trifluoromethoxy)phenothiazine) and the prototypic FMO3 substrate naphthalene. Preliminary activity data indicates that the D132H mutation renders the enzyme virtually inactive.

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ROLE OF HUMAN FLAVIN MONOOXGENASES IN THE SULFOLYSIS OF ALDICARB.

Environmental Toxicology Program, University of California, Riverside, Riverside, CA; Molecular Biology, Human Biomolecular Research Institute, San Diego, CA and Medicinal Chemistry, University of Washington, Seattle, WA.

Biotransformation of the carbamate insecticide, aldicarb, to aldicarb sulfoxide has been shown to significantly elevate its anticholinesterase activity. However, it is unclear what enzymes are responsible for this bioactivation. In this study, 51 human liver microsomal samples were prepared and aldicarb sulfoxidation was examined in vitro under saturating substrate incubations (200 uM). NA-DPH-catalyzed sulfoxidation activities were extremely variable and observed in 15 of 51 samples with a mean value of 0.034 ± 0.17 pmol/mg/min. Coincubation with 0.1% labrol to inhibit contributions of cytochrome P450 was not always effective (0.036 ± 0.26 pmol/mg/min). Bensydine N-oxidation was also examined and was 2.57 ± 1.66 nmol/mg/min and observed in all samples. FMO3 content was determined in all samples and demonstrated a significant correlation with benzidine N-oxidation (r2=0.41), but FMO3 did not correlate with aldicarb sulfoxidation. Incubation of aldicarb with 5 recombinant flavin monooxygenase isoforms indicated catalysis by FMO1 + FMO3, with no activity detected from FMO4 or FMO5. These results indicated limited hepatic biotransformation of aldicarb in human liver microsomes, but suggests other tissues may be more important such as fetal liver, the intestine or kidney which possess FMO1.

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PHARMACOKINETIC INTERACTIONS BETWEEN PYRIDOSTIGMINE BROMIDE (PB) AND PERMETHRIN IN RATS.
A. W. Abu-Qare, A. A. Abdel-Rahman and M. B. About-Dania.

Pharmacology and Cancer Biology, Duke University Medical center, Durham, NC.

A single oral dose of 13 mg/kg of PB, and a single dermal dose of 1.3 mg/kg of permethrin, alone and in combination were applied to male rats. Other animals received an intravenous dose of 1.3 mg/kg of PB or 1.3 mg/kg of permethrin. Five rats were sacrificed at 0.5, 1, 2, 4, 8, 24, 48, and 72 hours after dosing. In rats treated with a single agent, 0.5 and 72 hours after dosing, the application site retained 95% and 12% of PB, and 62% and 4% of permethrin respectively, and PB was not detected in the brain. Plasma peak concentrations were detected within 2 hours for PB (1.25 ng/mL), and within 24 hours for permethrin (193 ng/mL). Bioavailability of PB and permethrin was 28% and 52%, respectively. N-methyl-3-hydroxypridostigmine bromide was the metabolite of PB detected in plasma after 2 hours. Permethrin metabolites m-phenoxbenzyl alcohol, m-phenoxbenzyl alcohol and m-phenoxbenzyl acid were identified in plasma, liver and kidney 24 hours after administration. In urine, PB and its metabolite Nmethyl-3-hydroxypridostigmine bromide were identified. Urine contained m-phenoxbenzyl alcohol and m-phenoxbenzyl acid following application of permethrin. A total of 30% of the urinary excretion of permethrin was in the form of glucuronide and sulfate conjugates. Plasma concentration curves for PB and permethrin were fitted to a two and one compartment pharmacokinetic model, with a terminal half-life of elimination of 3.2 hours and 32.6 hours for PB and permethrin, respectively. Following combined intravenous dose of PB and permethrin, PB significantly increased Cmax, AUCplasma, T1/2, and decreased concentration of urinary metabolites of permethrin by 40%, 32% and 22% respectively. Also, Cmax, AUCplasma of permethrin was significantly increased (26%, 32%) and T1/2 was prolonged. The results show that PB significantly altered the pharmacokinetic profile of permethrin by interfering with its metabolism and excretion. This is due to competition for metabolism enzymes. This work was supported in part by the US army Medical Research and Materiel Command under Contract DAMD 17-99-1-9020.

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COMPARISON OF RATES OF GLUTATHIONE (GSH)-CONJUGATION OF TRITALOMETHANES.

Curriculum in Toxicology, UNC, Chapel Hill, NC and ORDINHEERL, USEPA, Research Triangle Park, NC. Sponsor: M. Evans.

Investigation into the mutagenic potencies of trihalomethanes (THMs; a class of water disinfection-by-products), mediated by GSH S-transferase (GST) theta, have described an association between a THM’s bacterial mutagenicity and its degree of bromination. Tested halomethanes ranked as follows in mutagenic potency: CICHBr = (CH3)2CHCl >> BeCICHCl = CH2Cl2, CHCl3 was not mutagenic at doses comparable to mutagenic doses of BeCICHCl. To enable a mechanistic understanding of these effects, we characterized GSH conjugation rates to compare this data with bacterial genotoxicity results. Several in vitro kinetic assays have been performed using THMs, CH2Br, or CH2Cl2 and GST theta. Using a gas-phase equilibration technique, loss of parent compound from the head-space (determined by GC-ECD) did not reveal differences between enzymatic and non-enzymatic rates for each THM, nor were differences in conjugation rates found when different THMs were compared. Similarly, no apparent rate trends were observed among the THMs when formaldehyde (HCHO) production was measured by the NADH assay. In contrast, CH2Br2 produced significant amounts of HCHO at equivalent concentrations. A more sensitive coupled assay that indirectly measures HCHO formation resulted in lower rates for the following THMs (units: nmol/min/mg protein *mM THM): BeCICHCl, (0.22), CICHBr, (0.23), CHCl3, (0.05), CH2Br2 (0.05). HCHO does not appear to be responsible for the measured bacterial genotoxicity as rates of HCHO formation from BeCICHCl and CICHBr were not detected, while the HCHO formation rate from CH2Cl2 was 20-fold greater than for BeCICHCl. That CH2Cl2 and BeCICHCl are equally potent in terms of mutagenicity, yet have significantly different rates of GSH conjugation and HCHO production, argues for existence of two separate pathways responsible for bacterial genotoxicity of di- and trihalomethanes; suggesting THM-dosed intermediates are more DNA-reactive and/or produce adducts that are more mutagenic. (Abstract does not reflect EPA policy).

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7H-BENZO[c]FLUORENE IN VITRO METABOLISM.


BENZO[c]FLUORENE (B[c]F) is tumorigenic in lung when administered to A/J mice. The mechanism by which B[c]F induces lung tumors is currently unknown, however, it is likely that the formation of B[c]F-DNA adducts play an important role in lung tumour induction. This study evaluates the in vitro metabolism of B[c]F in order to better understand the ability of B[c]F to form DNA adducts. A standard in vitro metabolism system using Aroclor induced rat liver 59 was employed. Metabolites were isolated using exhaustive ethyl acetate extraction and subsequently analyzed by reverse phase HPLC equipped with UV detection. The effect of reaction time and B[c]F substrate concentration were also evaluated. The 7-hydroxyB[c]F, 3-hydroxyB[c]F, and the 3,4-dihydrolitroB[c]F metabolites were synthesized and used to characterize the oxidation products observed with B[c]F. At least 7 oxidation products of B[c]F were detected within a 60 minute reaction time. The HPLC retention time of 7-hydroxyB[c]F corresponded to a predominant B[c]F oxidation product. When reaction times were increased to 160 min this metabolite peak was further converted to a more polar derivative. These results demonstrate that B[c]F is metabolized to a range of oxidation products in vitro and that the 7-hydroxyB[c]F metabolite can be further converted to a more polar derivative. These results, combined with recent DNA binding studies indicating that 7-hydroxyB[c]F is limited in its ability to form DNA adducts when applied to mouse skin. This suggests that the formation of 7-hydroxyB[c]F is a detoxification product of B[c]F. (This research was supported from EPRF).

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MECHANISM(S) OF DIFFERENTIAL INHIBITION OF HEPATIC AND PANCREATIC FATTY ACID ETHYL ESTER SYNTHASE BY INHIBITORS OF BETA ESTERASES.
B. S. Karpalia and G. S. Ansari.

1. Pathology, University of Texas Medical Branch, Galveston, TX and 2. Pathology, University of Texas Medical Branch, Galveston, TX.

In previous studies, we reported that hepatic fatty acid ethyl ester synthase (FAEES) activity compared to pancreatic FAEES is inhibited by tri-0-tolylsphosphate (TOTP), and that metabolism of TOTP is prerequisite for inhibition of FAEES as well as esterase activity. We also found that FAEES/esterase activity is associated with carboxylesterase (CE) in the liver and cholesterol esterase (CHE) in the pancreas. To further understand the interrelationship between hepatic and pancreatic FAEES, we synthesized two active metabolites of TOTP: 2-0-(crenale)4H-1,3,2-benzodioxaphosphoran-2-one (CBDP); cyclic sulfaglinate phosphinate and di-0-tolyl-4H-1,3,2-benzodioxaphosphuran-2-one (hydroxy-TOTP). Inhibitory effect of CDP and hydroxy-TOTP were studied using rat post nuclear (PN) fractions of hepatic and pancreatic homogenates, and commercially available PN fractions of hepatic and pancreatic Che. FAEES as well as esterase activity of hepatic PN fraction, and commercial CE and CHE was inhibited by CDP and hydroxy-TOTP in a dose-dependent manner using 0.01, 0.1 and 1 mg/ml concentrations. No inhibition of the FAEES/esterase activity was observed in the pancreatic PN fraction after
exposure to either TOTP metabolites. Both the activities in the hepatic PN fraction and commercial CE and GSH preparations by TOTP metabolites were found irreversible. Although, the differential effect of the TOTP metabolites on hepatic and pancreatic FAES/esterase activity is in agreement with the results of our earlier in vivo and in vitro studies, the anomaly of the differential effect on pancreatic FAES/esterase activity in the PN fraction and commercial CE/peptide preparation by the metabolite is not understood. Therefore, further studies are in progress for identification of cova lent adducts of FAES and metabolic products of CBP® and hydroxylated TOTP.

1121 HEPATIC GLUTATHIONE (GSH) CONJUGATION OF COUMARIN 3, 4-EPOXIDE (CE) IN MICE, RATS AND HUMANS.


1. CPS, The Procter & Gamble Co., Cincinnati, OH.

2. DuPont Pharmaceuticals, Newark, DE.

GSH, a reactive metabolite of coumarin, is thought to mediate coumarin-induced hepatotoxicity in rats. In the absence of GSH, CE spontaneously rearranges to 4-hydroxycoumarin (HOCO), which is also hepatotoxic. Metabolic activation of CE to GSH is highest in mice, a species that shows no evidence of hepatotoxicity, thereby suggesting that detoxification reactions are likely to determine species differences in liver toxicity. The purpose of the present work was to characterize hepatic GSH conjugation of CE in rats, mice, and humans. The GSH conjugate (CE-SG), formed at the 3-position, was isolated and used as a standard in an HPLC assay to quantify CE-SG. Non-enzymatic GSH conjugation was observed, accounting for approximately 45% and 80% of total CE-SG formed in mouse and rat liver, respectively. Glutathione S-transferase (GST)-catalyzed formation of CE-SG was observed in rat and mouse liver cytosols, with the Vmax for this reaction slightly higher in mice than rats (2.3 nmol/min/mg and 1.7 nmol/min/mg, respectively). Rat and human recombinant GST enzymes of the alpha and mu class catalyzed formation of CE-SG. The rat A1-3 and human A1-1 had the lowest Km values, while the highest activities were observed with the rat M2-2 and human M1-1. However, no GST-catalyzed formation of CE-SG was observed in human liver cytosols. It suggests that the formation of e-HPA is favored over CE-SG in humans. These results suggest that detoxification of CE by GSH conjugation is not the major determinant of species sensitivity to coumarin hepatotoxicity and implicates the detoxification of e-HPA as the critical detoxification pathway.

1122 INFLUENCE OF MATRIGEL-OVERLAYER ON CONSTITUTIVE AND INDUCIBLE EXPRESSION OF 9 GENES ENCODING DRUG METABOLIZING ENZYMES IN PRIMARY HUMAN HEPATOCYTES.


1. Environmental Health, University of Washington, Seattle, WA.

2. Pathology, University of Pittsburgh, Pittsburgh, PA.

Previous studies have shown that rodent hepatocytes overlaid with matrigel maintain expression of drug metabolizing enzymes, and restore the in vivo-like induction response of cytochromes P450 (CYPs). However, whether matrigel provides similar advantages for xenobiotic metabolism studies in primary human hepatocytes is uncertain. Therefore, the study evaluated the influence of matrigel-overlayer on the constitutive, phenobarbital (PB) and oltipraz (OPZ)-inducible gene expression in primary human hepatocytes. Hepatocytes were isolated from 5 viable human livers that were rejected for transplantation for various reasons. Hepatocytes were maintained in hormone-supplemented William's medium on a rigid collagen substrate overlaid either with matrigel or culture media alone, and treated for 48 h with 0.5 and 2 mM PB and 10 and 50 μM OPZ. The mRNA levels of 4 CYPs (1A1, 1A2, 2B6, 3A4) and 5 glutathione S-transferases (GSTs; A1, A2, M1, P1, T1) were determined by real-time RT-PCR. Protein levels of selected enzymes were assayed by immunoblot and activity analyses, following PB treatment. mRNA expression of CYP2B6, 3A4, GSTA1/2/3 was increased. OPZ induced mRNA levels of CYP1A2, 2B6, GSTA1/2/3. Our findings demonstrate further, that in the majority of cases, human hepatocytes overlaid with matrigel showed markedly higher constitutive and inducible gene expression relative to hepatocytes with no matrigel. Furthermore, the constitutive expression of most of the genes in hepatocytes overlaid with matrigel was similar to those in corresponding fresh liver tissue, and higher than hepatocytes with no matrigel. These results demonstrate that matrigel overlay facilitates the maintenance and induction of xenobiotic metabolizing enzymes in primary cultures of human isolated hepatocytes. (Supported by Hochschulunterprogramm III (DAAD) and NIH grants R01 ES05780 and P30 ES07033)

1123 MODELING GENETIC POLYMORPHISMS OF ALCOHOL DEHYDROGENASE.

J. G. Suliuotes 1, E. L. Flynn 2, and C. M. Pasto 3.

1. Pharmacology and Physiology, New Jersey Medical School, Newark, NJ.


3. Louisiana State University, Louisiana, LA.

The family of cytosolic enzymes known as alcohol dehydrogenase (EC 1.1.1.1) catalyze the reversible oxidation of alcohol to acetaldehyde, with corresponding reduction of NAD+ to NADH. Alcohol dehydrogenase in humans exists as multiple forms, including class and isozyme multiplicity, as well as allelic variations within several different isozyme forms. In the current study continuous system models were constructed to simulate the activities of those forms of alcohol dehydrogenase that play a major role in the biotransformation of ethanol. Kinetic macromoments reported in the literature were utilized to calculate microconstants descriptive of the Theorell-Chance mechanism for alcohol dehydrogenase homomers. Models for the C1 and C2 homomers, and the B1 homomer included the previously reported negative cooperativity and substrate inhibition, respectively. Homomer models were modeled as the sum of the activity of the respective monomers. All model re- indicated that under equilibrium conditions the reaction favored markedly the reduction of acetaldehyde to ethanol, as has been reported in the literature. In addition, comparisons of simulation output with published data indicated that the models accurately simulated the in vitro metabolism of ethanol by alcohol dehydrogenase. Interestingly, sensitivity analyses indicated that dissociation of NADH from alcohol dehydrogenase is not always the rate limiting step for the biotransformation of ethanol. These studies represent the first step in the investigation of how variability from genetic polymorphisms can be incorporated into biologically based models useful for risk assessment. (Supported by a grant from The American Chemistry Council).

1124 METABOLISM OF MENTHOFURAN IN F344 RATS.

L. C. Ferguson, E. H. Leberkin, and L. J. Barka. NIEHS, Research Triangle Park, NC.

Menthofuran is a major liver microsomal metabolite of pulegone, the chief component of pennyroyal oil. It has been detected in plasma of rats after i.p. administration of pulegone. Up to half of the pulegone hepatotoxicity has been estimated to arise from menthofuran. Menthofuran is further metabolized by liver microsomes to mimilactones through a reactive enol that binds to proteins. However, previous metabolism studies on pulegone and menthofuran in rats did not report common urinary metabolites derived from this pathway. Recently, we have identified previously unknown phase II urinary metabolites of pulegone. In order to determine the pulegone metabolites derived from menthofuran, a study of 14C-labeled menthofuran in F344 rats at a 60 mg/kg dose was conducted. Urine samples (24 h) were separated by HPLC equipped with a photodiode array detector and analyzed by MS. The urinary metabolite profile of menthofuran is complex. Two of the major metabolites were identified as 7α-hydroxy-3, 6-dimethyl-5, 6, 7, 7α-tetrahydro-2(4H)-benzofuranone (E3) and 7α-hydroxy-3, 6-dimethyl-3, 4, 5, 6, 7, 7α-tetrahydro-2-benzofuranone (G2) through comparison of their molecular weights, HPLC retention times, and UV absorption with those of the corresponding urinary metabolites of pulegone. Identification of common urinary metabolites from menthofuran and pulegone provides evidence for the in vivo metabolism of pulegone to menthofuran, then to mimilactones.

1125 IN VITRO METABOLISM OF CARBARYL BY HUMAN LIVER MICROSONES AND HUMAN CYTOCHROME P450 ISOFORMS.

J. Tang, Y. Cao, R. L. Rose and E. Hodgson. Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.

Carbaryl is a widely used carbamate pesticide. Its effect as a pesticide results from anticholinesterase activity. The present study was designed to investigate the metabolic activity of human liver microsomes (HLM) and human cytochrome P450 (CYP) isoforms toward carbaryl in vitro. Carbaryl was incubated with HLM or a CYP isoform at 37°C for 15 min. The 3 major metabolites, i.e, 5-hydroxy carbaryl, 4 hydroxy carbaryl and carbaryl methylol, were analyzed by HPLC. The Km and Vmax for 5-hydroxy carbaryl in HLM were 351 μM and 0.04 nmol/mg protein/min, for 4-hydroxy carbaryl were 421 μM and 0.9 nmol/mg protein/min, for carbaryl methylol were 72 μM and 0.53 nmol/mg protein/min. Using the same experimental conditions, the Km and Vmax in rat liver microsomes were 96 μM and
bioactivation in human lung cancers is probably catalyzed by hemoglobin and that LOXs and myeloperoxidase are not involved in NNK metabolism (Supported by CIHR #10382).

1128 THIODIGLYCOL METABOLISM BY ALCOHOL DEHYDROGENASE USING NMR: TWO-STEP OXIDATION PRODUCES 2-HYDROXYETHYLTHIOACETIC ACID AND 2 MOLES OF NADH.

A. A. Brimfield, D. A. Sarto1 and M. J. Nosvold, 1Biocatalysis, Pharmacology, USAMRICD, Aberdeen Proving Ground, MD and 2Experimental Therapeutics, WRAIR, Silver Spring, MD.

Sulfur mustard is a vesicant causing delayed injury to human skin. In aqueous medium mustard undergoes rearrangement and hydrolysis to form 2,2'-bis hydroxethyl sulfide (TDG), a dihydric alcohol. TDG is able to inhibit protein phosphaestes in cytosolic preparations but not the pure enzymes. Therefore, we are investigating metabolic activation. We demonstrated the metabolism of the compound by alcohol dehydrogenases (ADH) from horse liver and human skin [Brimfield et al. 1998], J. Biochem. Molec. Toxicol. 12:361] and from cloned human ADH isoforms [Dudley, Brimfield and Winston. 2000. J. Biochem. Molec. Toxicol. 14:244] by monitoring NAD reduction using OD 340. That technique, however, provides little information about intermediates and products. Recent work with ADH using NMR showed the mechanism varies with increasing substrate chain length and that the mechanistic difference is transparent to spectroscopy [Oppenheimer and Hennman. 1993. Adv. Exp. Biol. Med., 372: 407]. We used Oppenheimer's NMR technique to study the fate of TDG acted upon by horse liver ADH. Work was performed on a Varian 600 MHz NMR. Samples were prepared in 0.1 M NaNO₂, pH 7.5 containing 10% D₂O. Spectra were obtained at 37°C at 8 min intervals. The data showed the appearance of a singlet at 3.19 ppm from the methylene protons on carbon 2 of the acetate. Integration provided concentrations of the reactants which allowed us to evaluate kinetics and stoichiometry. The results indicate that TDG is oxidized to 2-hydroxyethylthioacetic acid by horse liver ADH via a two-step process in which TDG remains in the active site while two moles of NAD are sequentially reduced. There was no release of intermediate aldehyde as evidenced by the absence of a signal from an aldehyde proton at low field, nor was the monosaccharide a substrate. The structure was confirmed by synthesis. Additional metabolism work awaits the availability of cloned human ADH isoforms.

1129 SPECIES VARIATION IN THE METABOLISM, BIOACCTIVATION AND CYTOTOXICITY OF CARBARYL IN VITRO.


Pesticides are, by their very nature, toxic. However, the metabolism of these chemicals to reactive metabolites may play a pivotal role in the pathogenesis of chemical toxicity. We have investigated the metabolism, bioactivation and cytotoxicity of carbaryl using human mononuclear leukocytes (MNLS) as targets for toxicity and hepatic microsomes from sheep, chickens and humans as the metabolite generating systems. MNLS, isolated from whole blood, were incubated with hepatic microsomes and 14C-carbaryl (3-100μM) for 2h at 37°C in the presence and absence of cofactors. Following incubation stable metabolites were assessed by radio-HPLC-MS, protein-reactive metabolites assessed by exhaustive solvent extraction of the micromolar protein and cell death assessed by trypan blue exclusion. In the absence of NADPH, carbaryl was, in part, hydroxylated to yield 1-naphthol. Protein-reactive metabolite formation was low, however direct cytotoxicity to human MNLS was evident. In the presence of NADPH there was a significant increase in both the rate and routes of metabolism. The major order of metabolite formation was chicken-sheep-man. The metabolite profile in all species was similar, with the exception that 1.5 di-OH and 5, 6 di-OH naphthalene were produced by chicken microsomes only. Significant bioactivation of carbaryl was also evident, the rank order of protein reactive metabolite formation being sheep-man-chicken. Cytotoxicity was also increased in the presence of NADPH, with the rank order of cytotoxic metabolite formation being sheep-man-chicken. The introduction of the biologically ubiquitous cytochrome P450, reduced glutathione, decreased both the bioactivation and cytotoxicity, whilst the anti-oxidant, ascorbic acid, decreased the cytotoxicity. In conclusion, hepatic microsomal metabolism of carbaryl may enhance its bioactivation and cytotoxicity. Hepatic microsomes from sheep, chickens and humans, species that may become exposed to this chemical, have been shown to catalyze these biotransformations.
IN VITRO METABOLISM OF DIETHYL TOLUAMIDE (DEET) BY HUMAN, MOUSE, AND RAT LIVER MICROSOMES, HUMAN CYTOCHROME P450 ISOMERS, AND INTERACTION OF DEET WITH CHLORPYRIFOS.

K.A. Luman, R.L. Rose, J.A. Goldstein, W.G. Taylor, A.A. Beachfield, and E. Hodgson. Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC;CHOP, Research Triangle Park, NC; and USAMRICD, Aberdeen Proving Ground, MD.

Metabolism of the insect repellent N, 2-diethyl-m-toluamide (DEET) by pooled human liver microsomes (HLM), rat liver microsomes (RLM), and mouse liver microsomes (MLM) was investigated. DEET is metabolized by cytochrome P450s (CYP) leading to the production of a ring methylated metabolite product, N-diethyl-m-hydroxymethylbenzyl acetate (HMBAC) and a N-deethylated product, N-diethyl-m-toluamide (ET). Both the affinities and intrinsic clearance of HLM for ring hydroxylation are greater than those for N-deethylation. Pooled HLM show significantly lower affinities (Km) than RLM for metabolism of DEET to either of the primary metabolites (BALT and ET). Among 15 cDNA-expressed CYP enzymes examined, CYP1A2, 286, 261, 3A10 (3A4), and 2E1 metabolized DEET to the BALP metabolite while CYP2A6, 2A5, and 2C19 produced the ET BALP metabolite. CYP2B6 is the principle cytochrome P450 involved in the metabolism of DEET to its major BALP metabolite while CYP2C9 had the greatest activity for the formation of the ET BALP metabolite. Use of phenotyped HLM demonstrated that individuals with high levels of CYP2B6, 3A4, 2C19, and 2A6 have the greatest potential for metabolism of DEET. Mice treated with DEET demonstrated induced potential to metabolize DEET. This suggests a role for DEET as a possible exposure to HLM. A 2.4 fold increase in the levels of the CYP2B family, increased hydroxylation, and a 2.4 fold increase in the metabolism of chlorpyrifos to chlorpyrifos-oxon, a potent anticholinesterase. Preincubation of human CYP2B6 with chlorpyrifos completely inhibited the metabolism of DEET. (This research was supported by US Army Cooperative Agreement DAMD 17-00-2-0008).

EFFECTS OF PREGNANCY AND DIET ON PULSATILE ETHANOL EXCRETION IN FEMALE RATS FED VICA TOTAL ENTERAL NUTRITION.

J.M. Badejo, D. Babalola, T. Eby, J. Sweeney, C. Metcado, M. Perry, C. Breathford, S. Skelnatt, and M. J. Innes. Pediatric, Arkansas Children's Nutrition Center, University of Arkansas for Medical Science, Little Rock, AR.

We have developed intragastric infusion systems in which ethanol (EtOH) containing diets are fed as part of a system of total enteral nutrition (TEN). This allows complete control of ethanol and calorie intake. In this model, blood and urine ethanol fail to attain a steady-state plateau with a frequency of 6-7 days. Virgin female rats continuously infused with 13 g/kg/d of EtOH at a caloric intake of 160 kcal/kg/d had mean peak urine EtOH concentrations (UEC) of 369 ± 25 mg/dl at 200 ± 31 mg/ml at 24 hr. When the integrated area under the UEC profile of 2677 ± 164 hr mg/dl, the caloric intake was increased to 220 kcal/kg/d, the EtOH peak was reduced to 2090 ± 31 mg/ml at 200 ± 31 mg/ml at 24 hr. When pregnant female rats were infused with EtOH at the same dose at 220 kcal/kg/d, the EtOH peak was reduced to 209 ± 31 mg/ml at 24 hr. Pregnant female rats fed EtOH diets also showed a reduced nutritional requirements (pregnancy). Peak EtOH values were further reduced to 76 ± 8 mg/ml with an area under the curve of 309 ± 16 (P < 0.05). Pregnant female rats fed EtOH diets also showed a reduced nutritional requirements (pregnancy). Peak EtOH values were further reduced to 76 ± 8 mg/ml with an area under the curve of 309 ± 16 (P < 0.05). Pregnant female rats fed EtOH diets also showed a reduced nutritional requirements (pregnancy). Peak EtOH values were further reduced to 76 ± 8 mg/ml with an area under the curve of 309 ± 16 (P < 0.05). These values were significantly higher than those for (peak) and 1392 ± 283 (area). These values were significantly higher than those for the virgin rats fed 160 kcal/kg/d. The EtOH peak was reduced to 220 kcal/kg/d, the EtOH peak was reduced to 209 ± 31 mg/ml at 24 hr. These data suggest that diet and pregnancy both affect ethanol metabolism and clearance (supported in part by AA08645 T.M.B. and AA12819 M.J.R.).

IN VITRO TO EX VIVO EXTRAPOLATION OF INTRACELLULAR BSP METABOLISM PARAMETERS IN RAT LIVER.

F. Foy, J.L. Gourhan, A. Soto, and J. Frusetz. 1Physiology, Wright State University, Dayton, OH; 2ManTech Environmental, APHUR/HEST Wright Patterson AFB, Dayton, OH; and 3Toxicology Division, APHUR/HEST Wright Patterson AFB, Dayton, OH.

The primary purpose of a mathematical model of the kinetics of a toxin is to extrapolate to conditions/species/where an experiment is impossible. If the mathematical model is a detailed PBPK model, it is theoretically possible to obtain most or all parameters using in vitro methods and a minimal number of whole animals. Here we report a study using both isolated perfused rat livers (the in vitro part) and S9 preparations from rat livers (the in vivo part) to compare the intracellular and extracellular metabolism of BSP. For the perfused rat livers, eight different combinations of BSP concentrations, recirculating at one blood pressure, and initial BSP concentrations were used. The extrapolation methods were successfully used to support extrapolation methods. The S9 extracellular protein binding parameters were taken from the literature. The expected results included not only metabolism studies to estimate Vmax of BSP, but also protein binding studies to estimate BSP concentrations. These data are used to provide a toxicity risk assessment from S9 conditions to intracellular perfused liver conditions. Perfusion liver study metabolism parameters

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were estimated to be Vmax = 36.1 mmol/shell/liver and Km = 0.018 μM. The S9 study Vmax for whole liver was -131 mmol/shell/liver and the apparent Km (before best fit with 2 binding sites, one site having a Bmax of (3.8 μm/g scale-up factor, 9/1 to factor) mm and a Kd of 1.7 μm, and the second having a Bmax of (27.5 μm/g scale-up factor) mm and a Kd of 150 μm. Applying binding values to the S9 metabolisation BSP for total S9 of 7.5 μm and a scale-up factor of 200. This study shows promise and presents issues that must be considered for proper extrapolation.

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13-WEEK DIETARY AND DEVELOPMENTAL TOXICITY STUDIES OF POLYDIETHYL-SILAOXANE (PDMS) IN RATS AND RABBITS.

W. H. Siddiqui, H. B. Bicci, B. C. Timplikus and H. G. Meeks, Dow Corning Corporation, Midland, MI; Huntington Life Sciences, East, United Kingdom and Will Research Laboratories, Ashland, OH.

Potential hazards of two PDMS fluids, 10 and 350 cSt were evaluated in studies which included 13 week dietary and developmental toxicity studies in rats and rabbits. Purina® certified Chow mixed with the respective test articles at concentrations up to 10000 ppm for 13 weeks. In the developmental toxicity studies, the test article was administered by dermal route. 7 weeks of age, letter to gestation day 5 to 19 after mating at dosages of 300, 3000, and 30000 ppm. Animal administration in the feeding studies produced yellow oral application of the agonal region of males and females in the 5000 ppm group. Adverse effects of the test article include: non-nutritive test article in the diet and dose related increases in the corn ears and the site of the test article in the eyes. In the developmental toxicity studies, one female treated with 10000 ppm and one female in the control group had total litter mortalities. The incidence of these findings were not indicative of a test article effect. Litter parameters were considered to be unaffected and fetal examination revealed a number of abnormalities in both the control and treatment groups; however, none were considered to be attributable to treatment with PDMS fluids. Supported in part by Silicones Environmental and Health Safety Council of North America.

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MULTIGENERATIONAL 5-aza-2'-deoxyctydine ABNORMAL DEVELOPMENT.

F. Cucumer and S. Brande, Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.

Genotoxic imprinting plays a critical role in fetal growth and behavioral development. This is regulated in part by methylation of cytosines in DNA. 5-aza-2'-deoxyctydine (aza-2') is a potent inhibitor of DNA methylation thus controlling gene expression. Changes in methylation patterns can lead to heritable alterations in gene expression leading to abnormal growth and/lor morphological development. It has been suggested that epigenetic defects in germ cells due to loss of methylation-offspring. The aim of this study was to determine if the DNA demethylation takes place at the zygote stage of a mouse embryo. The study was designed in which three generations were examined. At gestation day 0 mice were observed and sacrificed 10 days after birth. Male and female mice were killed and used for fetal necropsy and skeletal analysis. In each generation, 50% of the offspring were allowed to give birth to produce the next generation. Litter size and abnormalities, abnormal male mating behavior, and retarded postnatal body development were observed in F1 a-2-deoxycysine offspring. In F2 generation, retarded females and delayed body development were observed. At birth, there was an abnormal ossification of the vertebrae, supernumerary thoracic ribs, reduced ossification of the sterna and increased number of fibro-cartilaginous attachments were statistically different when compared to controls. The F3 generation, males mated, reduced ossification of the vertebrae, supernumerary thoracic ribs, reduced ossification of the sterna and increased number of fibro-cartilaginous attachments were statistically different when compared to controls. Our data suggest that altered methylation patterns (induced initially by a-2-deoxycysine exposure) may be inherited by the offspring of exposed parents leading to altered imprinting and development.

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CONTROL VAGINAL OPENING DATA FOR F1 GENERATION CRL SPRADE-DAWLEY ("GOLD STANDARD") RATS IN EPA DEVELOPMENTAL NEUROTOXICOLOGY, EPA MULTIGENERATION AND FDA PERI-POSTNATAL STUDIES.

A. M. Hoberman, M. S. Clamirn, E. Lewis and J. Barnett, Jr., Argus Research, A Division of CRL, Harsham, MA.

Vaginal opening and baloon-preparatal separation are classically considered to be onendocrine-mediated developmental endpoints indicative of sexual maturation in the rat that are generally independent of body weight. Because these events do not occur until after postnatal day 21, the usual day rat pups are weaned, the reported values are usually based on observations for 1 male and 1 female weaning rat per litter. Currently, there is considerable controversy regarding the adequacy of using one pup/sex/litter to represent the litter, because it has been shown for body weights reflecting random selection. However, historically, one weaning rat/litter has been commonly employed in EPA-mandated Developmental Neurotoxicology and MultiGeneration Studies, in as well as in FDA-compliant Peri-Postnatal Studies, to identify whether evaluating additional animals was not only practical, but also Developmental Neurotoxicology Studies and compared these individual and mean/litter data with that obtained in three MultiGeneration Studies and seven vaginal opening ranges from day 31.6 to day 34.0, based on 60 rats/group (3 litter, 20-Postnatal Studies) is evaluated, the mean day for vaginal opening ranged from cageing that statistically significant differences may be associated with random selection of animals (individual values were investigated). The mean/litter data were found to be generally analytical and body weight, reflecting random selection. It should be noted that statistical significany differences may be associated with random selection.

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VALIDATION OF THE MORRIS MAZE AS A TEST OF LEARNING AND MEMORY IN YOUNG RATS - SUITABILITY FOR DEVELOPMENTAL TOXICITY STUDIES.

S. D. Renaux, D. P. Myers and M. J. Collier, Toxicology, Huntington Life Sciences, Huntington, United Kingdom, Sponsored: C. Anderson.

To assess the suitability of the Morris water maze as a test of learning and memory in young rats, performance of CD rats was assessed at 4 and 9 weeks of age after administration of Scopolamine, Groups of 10 male and 10 female rats were dosed by intraperitoneal injection with Scopolamine hydrobromide at 1.5 mg/kg/day on each of days 28-30 or 63-65 of age. Twenty minutes after each dose Bob was placed individually in the Morris water maze - a circular pool (0.9 m diameter) filled to a depth of 30 cm with water at constant surface temperature of 24°C. Each animal received a series of trials on each of 3 consecutive days of testing. In each trial, the rat was placed in the water at the perimeter of the pool and allowed a maximum of 90 seconds to find the platform. The time to reach the platform and the number of pool sectors crossed was recorded. Treatment of rats with Scopolamine on days 28-30 and Days 63-65 resulted in a general impairment of performance in the water maze - for both sexes there were some statistically significant increases in mean trial times and mean Controls, which showed improved performance over the 3 days. Control rats were tested for performance over the 3 days of testing, performance of treated males and females on Days 63-65 of age showed improved performance over the 3 days of testing but females showed no improvement. These results demonstrate the suitability of the Morris water maze for assessing learning ability on rat developmental toxicity studies.

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THE USE OF THE MOUSE FOR EVALUATION OF PREGNATAL DEVELOPMENTAL TOXICITY AND REPRODUCTION AND FERTILITY EFFECTS.

M. E. Moonon and R. W. Lewis, NIEHS, Syngenta CRL, Macclesfield, United Kingdom, Sponsored: E. Lock.

In regulatory toxicology, the rat is the guideline preferred species for evaluation of prenatal developmental toxicity and for evaluation of reproduction and fertility effects. However, in some instances the rat may not be the most appropriate model i.e. when the test is the most relevant species for human health risk assessment. Since the mouse has been developed successfully as an alternative rodent.
1140 CONTROL PRIMORDIAL FOLLICLE COUNTS IN MULTIGENERATION STUDIES IN CRL. SPRAGUE-DAWLEY ("GOLD STANDARD") RATS.

M. S. Christian1 and W. R. Brown1, Argus Research, A Division of CRL, Harsham, PA and 2Research Pathology Services, New Britain, PA.

Ovarian primordial follicle (PF) count is an endpoint in the 1998 EPA multiagen. PF counts are identified for ovaries from at least 10 F1 control and high group female, 5 sections/ovary. Mean/ovary are determined and compared, lower group and assay sensitivity are associated with age, number of animals, sections/ovary, section thickness, and criteria determining PF. Generally, 4 PF grades (1, 2, 3a, 3b) are counted (oocyte without surrounding granulosa cells to oocyte ringed by granulosa cells). These criteria were used in 3 multigeneration EPA- and GLP-compliant studies at our laboratories, using the same rat strains, procedures, definitions and evaluator. Variation existed in control PF counts between sites and left ovaries of the same rat, mean values for individual ovaries and mean values/group. We have heard that our data differs from that of other groups. In two, findings indicate that intraspecies laboratory differences exist, and that currently performed, this endpoint may be not sufficiently reproducible to be considered a pivotal endpoint for human risk assessment. In our 43 studies, the response of the PF counts was not controlled. Were based on 30 rats/group, in the 3rd study, values were based on 10/group. We confirmed age-related differences between P and F1 rats in 1 study (mean/ovary: P = 9.29 + 5.32 and 8.89 ± 3.48; F1 rats = 14.50 ± 6.12 and 16.07 ± 5.75). Mean values/ovary for F1 rats in the 3 studies for each ovary = 14.50 ± 6.12 and 16.07 ± 5.75, 13.36 ± 7.44 and 13.41 ± 7.49, and 13.64 ± 8.36 and 12.66 ± 8.42. Mean PF values/ovary ranged from 2.6 to 44.4. Mean PF of 26 = 1, 1, 1, 3 and 7 PF5 sections: control/ovary mean PF = 4.8 (9, 3, 2 and 11 PF5 sections). Mean PF of 44 = 51, 75, 25, 54 and 17 PF5 sections: the control/ovary mean PF = 26 (29, 27, 23, 27 and 24/5 sections). Individual values, criteria and artifact types will be shown.

1141 REPRODUCTIVE EFFECTS OF 1, 2, 3, 4-BUTANE-TETRACARBOXYLIC ACID (BTCA) IN SPRAGUE-DAWLEY RATS WHEN ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL.

K. Layton1, G. Wohle2, Y. Wang1, J. Quanece3 and J. Bishop.1. The Immune Research Corporation, Cazenovia, MD; 2AM, Frederick, MD and 3NIEHS, Research Triangle Park, NC.

BTCA was evaluated using the multigenerational Continuous Breeding paradigm to reveal potential reproductive toxicity in rats. Beginning on Study Day 1, BTCA was administered daily via oral gavage at doses of 0, 100, 250, 500 mg/kg/day to adult male and female rats (N=20). Mating pairs were to produce three litters (F1, F2, and F3). Dosing of the F1 group (F1 animals) was initiated on post-natal day (PND) 22. On PND 81-10, F1 (N=20) animals were assigned to mating pairs and allowed to produce three litters (F2, F3, and F4). Endpoints evaluated included body weight, food consumption, clinical signs, reproductive/fitness parameters, sexual development and pathology. No changes were seen in the body weights or food consumption of the F2 animals. Decreases of 4-5% were seen in the body weights of the 500 mg/kg group for males followed by a decrease in body weights of the 500 mg/kg group for females. The decrease in body weight was observed during Week 12 for the 100 mg/kg males. Throughout the F2 and F3 generations, most reproductive parameters were unchanged in all dose groups when compared with the control and with the exception of decreases in the pregnancy indices in the 500 mg/kg groups compared to control. Control for the second litter of each generation (F3) was compared to 20/20, F3, 15/17 compared to 20/20, F3, 15/20 compared to 20/20. Sperm parameters and gonadal histology were unchanged in the F3 and F4 generations. The remaining reproducitve changes were not considered to be treatment-related. The only microscopic lesion related to treatment was a renal lesion consisting of the presence of multinucleate giant cells, often centered around acinar crystalline material in both F2 and, at a decreased incidence, F3 generations. The incidence of renal lesions continued to increase with time of exposure and was greater than 50% at the 100 mg/kg dose level and had decreased by 50% at the 10 mg/kg dose level. The lesions were not considered to be a reproductive toxicant.

1142 ANTANDROGENIC EFFECTS OF A PITHALATE COMBINATION IN IN UTERO MALE REPRODUCTIVE DEVELOPMENT IN THE SPRAGUE-DAWLEY RAT: ADDITIVITY OF RESPONSE.

P. M. Foster, K. J. Turner and N. J. Barlow, CII Centers for Health Research, Research Triangle Park, NC.

Environmental antitrogens constitute a class of chemicals that significantly affect reproductive development in laboratory animals. In the environment it is more likely that exposure will occur to multiple agents that may have similar or different mechanisms of antitrodienicity. The objective of the present study was to examine the reproductive developmental effects on male rats exposed to two phthalates in utero, both as individuals and in combination. Dose levels were select based on LOAELs for reproductive development obtained from previous dose-response studies. Pregnant Crl:CD(SD)BR rats, 10 per group, were treated by gavage with either corn oil vehicle, 100 mg/kg/day of dibutyl phthalate (DBP) or o(2-ethylhexyl) phthalate (DEHP) or a combination of each phthalate at 100 mg/kg/day (in combination respectively 200 mg/kg/day of phthalate) on gestation days 12 to 21. On postnatal day (PND) 1 the anogenital distance (AGD) and male pup body weights were measured and each individual pup was uniquely identified with a footpad tattoo. On PND 13 the retained areole were counted on each male pup. At necropsy on PND 90 the AGD was again. Organ weights were recorded for the testes, epididymides, prostate (dorsal and ventral) and seminal vesicles. On PND 1 the AGD was reduced significantly in the combination group while it did not differ from control for DBP or DEHP alone. Significantly increased numbers of areole were retained on PND 13 in the males in the combination groups not in the other two phthalate groups. There were no consistent changes in organ weights and only a small number of reproducitve tract lesions were seen in the phthalate-exposed animals. In line with default assumptions, the effects of prenatal exposure to a combination of two phthalates on AGD and nipple retention in male rats was additive in comparison with the effect of exposure to each phthalate alone.

1143 PERMANENT ALTERATION OF ANOGENITAL DISTANCE AND NIPPLE RETENTION IN MALE RATS EXPOSED TO DIBUTYL PHTHALATE IN UTERO.

N. J. Barlow, B. S. McInroy and P. M. Foster. CII Centers for Health Research, Research Triangle Park, NC.

Dibutyl phthalate (DBP) has been shown to act as an antitrodienic when male rat fetuses are exposed during late gestation. Antitrodienic effects noted include decreased anogenital distance (AGD) on postnatal day (PND) 1 and increased retention of areole on PND 13. The objective of this study was to compare the AGD and areole/nipple retention data obtained during the early postnatal period to those obtained in adulthood to determine if the changes were permanent. pregnant Crl:CD(SD)BR rats, 10 per group, were treated by gavage with either corn oil vehicle or DBP at 100 and 500 mg/kg/day on gestation days (GD) 12 to 21. AT PND 1 the AGD and male pup body weights were measured and retained areole were counted on PND 13. These end points were also collected at necropsy on PND 180. Pups exposed to 500 mg/kg/day exhibited a significant, 14%, decrease in AGD on PND 1. At necropsy these DBP-exposed offspring exhibited a significant, 10%, reduction in AGD. The reduction in AGD at PND 1 correlated with the reduction in AGD at PND 180 (r = 0.30). Similar effects were seen in areole/nipple retention with significantly increased numbers of retained areole/nipples in the high dose group at both PND 13 and 180. Lesions in the male reproductive tract included aneureatic testes accompanied by hypoplastic or malformed epididymides, malformed seminal vesicles and small prostates. The incidence and the weight of these lesions were assessed. The reproductive effects of control animals were 2%, while at 100 and 500 mg/kg/day they were 9 and 89%, respectively. These data demonstrate that decreased AGD and increased nipple retention following in utero DBP exposure are permanent effects with strong correlation between the data from the early postnatal period and adulthood. As the alterations in AGD and areole/nipple retention are permanent, there is support for the argument that these end points are malformations and should be incorporated routinely into standard testing protocols and considered in risk assessment.

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1144 LACK OF DEVELOPMENTAL TOXICITY IN SD RATS WITH DI(2-ETHYHEXYL)TEREPHALATE (DEHT, DOPT).

A developmental toxicity study was conducted in Sprague-Dawley rats with DEHT. Groups of 25 mated females were exposed to 0, 0.3, 0.6, and 1.0% DEHT in the diet beginning on Day 0 of gestation until termination on Day 20. Mean body weight and body weight changes were reduced for the 1.0% group. Net body weight and body weight change were significantly lower for the 1.0% group compared with controls. On Day 20, dams were euthanized; the uterus was removed and weighed. The numbers of viable fetuses and early and late resorptions were counted. Each fetus was evaluated for external, visceral, and skeletal anomalies. No effects on litter size or mean pup weight were observed. There were no differences in the percentages of viable fetuses or early or late resorptions. No visceral or skeletal effects were seen that could be attributed to treatment. The NOAEL for developmental toxicity was 1.0% in the diet. These data complement the information from Gray et al. (2000) which indicate that DEHT does not produce antianogenetic-like effects, and the data from a 2-generation reproductive toxicity study indicating a lack of reproductive toxicity.

1145 EFFECTS OF IN UTERO FENITROTHION EXPOSURE ON ANDROGEN-DEPENDENT MALE RAT REPRODUCTIVE DEVELOPMENT: A DOSE-RESPONSE STUDY.

Fenitrothion ([O-Dimethyl-O-(4-nitro-m-tolyl)phosphorothioate]) is an organophosphate insecticide that is known to antagonize the androgen receptor. The objective of the present study was to evaluate the ability of fenitrothion to disrupt androgen-dependent sexual differentiation in the male rat. Pregnant Crl:CD(SD)BR rats were administered fenitrothion by gavage at 0.5, 10, 15, 20, or 25 mg/kg/day (n=6-11/group) from gestation day (GD) 12 to 21. Maternal toxicity was observed in the dams treated with 20 and 25 mg/kg fenitrothion/kg/day based on tremors and a significant decrease in body weight gain between GD 12 and 21. Embryo-fetal death was increased in the 20 and 25 mg/kg/day exposure groups as evidenced by a significant decrease in the proportion of pups born alive. In utero exposure to fenitrothion altered androgen-dependent sexual differentiation, confirming previous findings that fenitrothion is an androgen receptor antagonist in vivo. Anogenital distance (AGD) in the male offspring on postnatal day (PND) 1 was decreased relative to the control group by 8% (p<0.06) and 16% (p<0.001) in the 20 and 25 mg/kg/day dose groups, respectively, with body weight as a covariate. Fenitrothion exposure significantly increased areola retention in male rats on PND 13 from 1.25 ±0.46 in the control group to 4.31 ±0.66 areole per rat in the 25 mg/kg/day dose group (litter means ±SE). These alterations in AGD and areola retention were transient and not observed at sexual maturity (PND 100). Fenitrothion exposure did not induce a dose-dependent increase in the incidence of gross lesions of the male reproductive tract. Body weights and organ weights were unaltered in the adult male offspring. Fenitrothion exposure induced cholinergic stress in the dams and fetal mortality at the same dose level as that found to elicit the transient but significant effects on AGD and nipple retention in the male offspring.

1146 VALIDATION OF DEVELOPMENTAL NEUROTOXICITY ENDPOINTS IN RATS ADMINISTERED METHAZOLMUNE IN DRINKING WATER.

The purpose of this study was to validate procedures outlined in the USEPA OPPTS 870.6300 guideline for developmental neurotoxicity (DNT) studies. Pregnant Sprague-Dawley rats (N=25/group) were administered either 0, 10, 20, or 100 ppm methazolmune (used to treat human hypothyroidism) in drinking water from gestation day 6 through postnatal day (PND) 21. Endpoints evaluated in the dams included a modified functional observational battery (mFOB), body weights, and food and water consumption. In the pups, brain weights, qualitative neuropathology and quantitative brain morphometry were evaluated. PND 11, 22 and 72. Auditory startle, locomotor activity, learning and memory (Biel Maze) and mFOB were assessed in the offspring repeatedly throughout their development. During lactation, the dams in all three exposure groups weighed significantly less, and consumed less food and water than controls. Locomotor activity was reduced on PND 13 and increased on PND 21 for 30 and 100 ppm offspring relative to controls. Auditory startle response endpoints (Vmax, Tmax and Vave) were affected in the 30 and 100 ppm offspring at PND 20 and 60. Pup body weights were reduced from PND 1-70 for both sexes in all exposure groups relative to controls. Delayed sexual maturation (balanopreputial separation and vaginal patency) was readily apparent in all three exposure groups, except for vaginal patency at 10 ppm. In addition, on PND 11 and 22 both male and female offspring mean brain weights (100 ppm) were reduced relative to controls. Brain weights were reduced on PND 72 for 100 ppm male offspring only. No dam or offspring exposure-related deficits were noted in the mFOB at any time point. Qualitative neuropathology and quantitative brain morphometry at PND 11 (comparing immersion and perfusion fixation at PND 11, 22 and 72, along with learning and memory data, will be presented. The data analyzed thus far are being used to assess the appropriateness of methazolmune as a positive control agent for DNT studies.

1147 REPRODUCTIVE EFFECTS OF DIETHYLDIHYDROPHthalate (DEHP) IN SPRAGUE-DAWLEY RATS WHEN ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL.
G. W. Wolfe, L. Layton, I. Nehrebecky, Y. Wang, F. Chen1, S. D. Rouselle and J. Bishop. 1TheImmunotoxicology Research Corporation, Gaithersburg, MD, 2DaPonte Pharmaceuticals Company, Newtown, DE, 3PAI, Frederick, MD and 4NIEHS, Research Triangle Park, NC.

DEHP was evaluated using a multi-generational protocol in rats to assess potential reproductive effects over multiple generations. Beginning on Study Day 1, DEHP was administered to the diet at doses of 0.0, 10, 100, 300, 500, 1000, 3000, and 7500, and 10,000 ppm to adult male and female rats (N=17). Each mating generation was allowed to produce three litters (F1,F2, F3, F4, F5, F6, F7, F8). On PND 81 ±10, animals from the third litter from each generation were assigned to mating pairs (N=17). Reductions were noted in mean body weight of animals receiving 7500, 10,000, and 10,000 ppm DEHP. Reproductive/litter effects including decreases in pregnancy, indices for most generations; decreases in the no. of males per litter and the total no. of pups per litter; decreases in male and female pup weights at birth and during lactation; decreases in anogenital distance for male pups; and delay of sexual development parameters were noted at the top 2 dose groups. The 10,000 ppm animals did not produce any F2 generation animals. Outbreeding at 7500 and 10,000 ppm of treated males with naïve females revealed decreases in mating, pregnancy and fertility indices and decreases in the number of implantation sites. Sperm endpoints were decreased at 7500 ppm in all generations and at 10,000 ppm in the F2 and F3 generations. Organ weight changes of the liver (increase), kidney (increase), and male accessory sex organs (decrease) occurred at 1,000, 7500, and 10,000 ppm. Treatment-related microscopic findings were noted at 7500 and 10,000 ppm in the testes, epididymides, liver, adrenal, and kidney in all generations and in the liver at 1,000 ppm in the F2 and F3 generations. DEHP is considered a reproductive toxicant at 7500 and 10,000 ppm. There was no general or reproductive effects observed at dose levels at or below 1,000 ppm except liver toxicity at 1,000 ppm.

1148 DECREASE IN THE LITTER SIZE OBSERVED IN FEMALE RATS INTRAVENOUSLY GIVEN L-CYSTEINE.

There are few reports on the reproductive and developmental toxicity of amino acids. We previously observed marked decrease in the litter size in female rats intravenously received a high dose of L-Cysteine (CyS) before mating. In the present study, we investigated the oocytes morphologically before and after mating to clarify the cause of the decrease in the litter size observed in the rats. SD rats, 11 weeks old, were intravenously infused CyS at a dose of 1000 mg/kg for more than 1 week before mating. The unfertilized oocytes (UO) collected from ampulls of uterine tubes and fertilized embryos (gestation day (GD) 1 and 2) collected from oviducts were examined morphologically by stereomicroscope. Morphometry was also performed for UO. The number of females in the CyS group from which UO could be collected was the same with that in the control group (non-treatment group), but the number of observed oocytes was a half of that in the control group. Moreover, zona pellicuda (ZP)-lacking or partially ZP-lacking oocytes were observed in the CyS group. The morphology of UO in the CyS group showed similar diameter as compared with that in the control group, but ZP became thin. The number of female rats from which embryos were collected on GD 1 in the CyS group was similar to that in the control group, but the number collected on GD 2 was reduced to a half of that in the control group. The number of observed embryos in dams on GD 1 and GD 2 were decreased markedly in the CyS group, and most of them were degenerative embryos. It was observed in this study that a high dose of CyS induced the lacking or partially lacking of ZP or thinning of ZP in UO and embryos on GD 1 and GD 2. It is known that ZP plays an important role in...
early embryogenesis. The oocytes, which were affected at ZP by CySH treatment, were degenerated. In conclusion, affected ZP was considered to contribute to the decrease in litter size.

1149 CONTROL PREPULTRAL SEPARATION DATA FOR F1 GENERATION CRL SPRAGUE-DAWLEY ("GOLD STANDARD") RATS IN EPA DEVELPOMENTAL NEUROTOXICOLGY, EPA MULTIGENERATION AND FDA PERI-PPOSTNATAL STUDIES.


Balano-preputial separation and vaginal opening are classically considered to be endocrine-mediated developmental endpoints indicative of sexual maturation in the rat that are generally independent of body weight. Because these endpoints do not occur until after postnatal day 21, the usual rat dam pups are weaned, the values are generally based on observations for one male and one female weanling rat/litter. Currently, there is considerable controversy regarding the adequacy of using one weanling rat/group to represent the litter, because it has been shown, for body weight and uterine weights, that this practice can result in statistically significant differences reflecting random selection. However, historically, one rat/litter has been the number evaluated for these parameters in EPA-compliant Developmental Neurotoxicology and Multigeneration Studies, as well as in FDA-compliant Perinatal Studies. To identify whether evaluating additional animals was not only practical, but also provided improved data sets, we evaluated 3 rats/group in Developmental Neurotoxicology Studies and compared these individual and mean/litter data with those obtained in 3 Multigeneration Studies and 7 Perinatal Studies. The results of these studies indicate: (1) The mean day of prepuberal separation ranged from days 43.2 to 48.4, based on 60 rats/group (3 litter, 7 Developmental Neurotoxicology Studies), (2) When 1 rat/litter (25 groups; 7 Peri-PPostnatal Studies) was evaluated, the mean day for vaginal opening ranged from 44.6 to 48.1, (3) Individual values varied markedly among control animals, indicating that significant differences observed between studies may be associated with random selection of animals to be evaluated (individual values ranged from postnatal days 40 to 76). (4) Use of 3 rats/group improved the sensitivity of the assay. (5) There appeared to be a correlation between the age at sexual maturation and mean age at GD 0.

1150 ROLES OF PROGESTERONE ON SUPRESSION OF UTERINE DECIDUALIZATION AND IMPLANTATION FAILURE INDUCED BY DIPHENTYLCHLORIDE (DPTC) IN RATS.

M. Inoue and E. Miyawaki. Osaka Branch, National Institute of Health Sciences, Osaka, Japan.

In our previous study, administration of DPTC at 16.5 mg/kg higher on days 0-3 of pregnancy was found to induce implantation failure in rats (Toxicol. Lett., 108, 17-25, 1999). In the present study, the effects of DPTC on uterine function, as a cause of implantation failure, and the roles of progesterone in DPTC-induced implantation failure were determined in rats. Female rats were given DPTC by gastric intubation at 3.0, 8.3, 16.5, and 24.8 mg/kg on days 0-3 of pseudopregnancy and the decidual cell response was determined on day 9 of pseudopregnancy. The uterine weight on day 9 of pseudopregnancy served as an index of the uterine decidualization. A significant decrease in the uterine weight, which indicates suppression of the uterine decidualization, was detected at 16.5 and 24.8 mg/kg. The ovarian weight and number of corpora lutea in the DPTC-treated groups were comparable to the controls. A significant decrease in the serum progesterone levels was found at 16.5 and 24.8 mg/kg. Hormonal regimen supported the decidual development in ovarioctomized rats given DPTC. The pregnancy rate and number of implantations in the groups given DPTC at 16.5 and 24.8 mg/kg on days 0-3 of pregnancy in combination with progesterone on days 0-8 of pregnancy were higher than those in the groups given DPTC alone. It could be concluded that implantation failure due to DPTC is mediated via the suppression of uterine decidualization correlated with reductions in serum progesterone levels.

1151 HOMOLOGOUS RECOMBINATION AS A POTENTIAL MECHANISM FOR PHENOTYP-IN-INDUCED TERATOGENICITY.

P.M. Kim and J. A. Nickoloff. Dept. of Pharmacology & Toxicology, and School of Environment Studies, Queens University, Kingston, ON, Canada and Dept. Mol. Genet. & Microbiol., Univ. of New Mexico, Albuquerque, NM.

Epilepsy treatment involves the use of anticonvulsants such as phenytoin (PHY). PHY is teratogenic in numerous species, but the mechanism by which it initiates teratogenicity remains to be elucidated. PHY can be enzymatically bioactivated to a reactive intermediate capable of damaging essential macromolecules including DNA. PHY can induce DNA double-strand breaks (DSB), which may be repaired through homologous recombination (HR). Increased levels of DSB may induce hyper-recombination that can lead to deleterious genetic changes. We hypothesize that these changes, such as gene deletions, may be an important molecular mechanism for chemical-induced teratogenesis. We used a Chinese hamster ovary (CHO) cell line with a neo direct repeat recombination substrate (CHO 3-6). The 5' neo is driven by the deoxymethionine (DMO) inducible metabolic virus, the second neomycin promoter. HR frequencies were determined by counting the number of G418-resistant colonies per live cell, and recombinant products were analyzed by Southern blot to distinguish gene conversions without an associated crossover from deletions, which can occur by crossing over or single-strand annealing. CHO 3-6 cells were treated with PHY at 80, 240 and 800 uM for 4 or 24 hr, and the 24 hr experiments were performed in the presence or absence of DEX. We found that PHY enhances HR in a dose-dependent manner, and that PHY-induced HR is further enhanced by increased transcription of the 5' copy of neo. Of more than 10 HR products analyzed to date, all arose by gene conversion without associated crossover. These results are similar to HR stimulated by 1-Scyl nucleoside-induced DSBs, but contrast with the high rate of deletions seen with spontaneous and UV-induced HR. Thus, our data suggest that PHY-initiated DNA damage induced HR proceeds through a DSB intermediate, implicating HR as a potential mechanism for chemical-induced teratogenesis.

1152 STUDIES OF EMBRYOTOXIC EFFECTS OF BITUMEN-SALT MASSES (BSM) AND THEIR AQUEOUS EXTRACTS CONTAINING GB, GD, OR VX TWO-STAGE DESTRUCTION PRODUCTS.


In support of Cooperative Threat Reduction activities, the Research Institute of Hygiene, Occupational Pathology and Human Ecology in St. Petersburg, Russia participated in joint studies with the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) to investigate the potential reproductive effects of materials leached from the bituminous-salt mass (BSM) end-products of the Russian two-stage chemical agent destruction process. These studies were conducted to address concerns that materials leached from the BSMs could pose a health threat to receptors in the vicinity of future storage/disposal sites. Using aqueous leachates or soil extracts derived from Russian bitumen alone, or BSM containing demilitarized Russian manufactured sarin (GB), soman (GD) or VX, embryotoxicity was evaluated in vivo using pre- and post-implantation rodent embryos cultured in blood sera. Embryos were exposed either directly to aqueous extracts or indirectly by using serum obtained from donor animals that were exposed orally to oil suspensions of bitumen or the various BSMs for different durations. Developmental anomalies included dimorphism, decreased cleavage rates, yolk sac edema, hemorrhages (pericardial sac, brain, allantois fluid) and decreased brain dimensions. Diluting extracts to 4% eliminated their embryotoxic action. In other studies, embryofetotoxic effects were seen in embryos of pregnant rats orally exposed to aqueous extracts of bitumen, GB, GD, or VX-BSM (4.20 or 100%) daily during gestational days 1-19. Embryos showed the highest sensitivity to BSM-VX aqueous extracts. In summary, BSM aqueous extracts possess weak embryotoxic properties that decrease proportional to decreasing exposures. While all BSMs demonstrate some measure of embryotoxicity, BSM-VX is the most toxic for embryonic development.

1153 THE EFFECTS OF CONTINUOUS INTRAVENOUS SALINE INFUSION UPON CLINICAL PATHOLOGY PARAMETERS IN THE WEANLING ALBINO RAT.


The use of pharmaceautics and biotechnology products to treat pediatrics requires the juvenile animal studies to be considered prior to conducting clinical trials. The weanling rat represents a suitable model for testing infusion products since it allows for the use of the clinical dose route in the pre-clinical testing. In this study, animals were cannulated at Day 22 post partum using routine surgical procedures and thereafter infused continuously with physiological saline at rates up to 5 mL/kg/h up to 43 days post partum. Terminal blood samples were collected on Days 37 and 44 post partum from the abdominal aorta and analyzed for a standard panel of hematology and clinical biochemistry parameters. There were only minor effects upon clinical condition and a general lack of, or minimal, local tissue damage or inflammatory reaction seen post-mortem at the infusion site in infected animals. Consequently, the clinical pathology data generated for these animals allowed for a direct evaluation of the potential changes associated with saline infusion. Although a slight reduction in white blood cell numbers was observed in infused animals versus age-matched historical controls, the values recorded for the infused weanlings.
remained within our laboratory reference ranges for older (8 to 12 week old) rats. The differences between the remaining clinical pathology parameters of infused senna treated animals were minor and likely represented biological variation. Consequently, it was concluded that continuous intravenous saline infusion did not adversely affect the clinical pathology of weaning rats thus confirming the suitability of this animal model for testing infusion products.

1154 CONTINUOUS INTRAVENOUS INFUSION IN THE WEANLING ALBINO RAT.

The use of pharmaceuticals and biotechnology products to treat pediatrics requires that juvenile animal studies be considered prior to clinical trials. The weanling rat represents a suitable model for infusion products since it allows for the use of the clinical dose route involved in the pediatric population. For over a decade, our laboratory has conducted developmental/repreaductive studies in rats and rabbits using the infusion route and recently applied our experience with infusion technology to weaning rats. Using aseptic techniques, 40 weanling rats were anesthetized and surgically implanted via the femoral vein with a medical-grade silicone-based catheter at 22 days of age. A perfusion apparatus that was suitably scaled for their size and were infused with physiological saline via an in-line 0.5 μm filter, for 24 hours/day, up to 42 days of age. Dosimetry data for the infusion rates employed (2.5 and 5.0 μl/h) confirmed animals received within ±10% of the nominal dose volume at each infusion rate over the course of the investigation. There were no systemic evidence of toxicity, body weight and clinical pathology parameters and these data are presented. The general lack of, or minimal, local tissue damage or inflammatory reaction seen pathologically at the injection site suggests that the infusion interval could be extended beyond 42 days of age, if required. In conclusion, weanling rat infusion provides an appropriate animal model to assess the safety and pharmacodynamics of infused drug products intended for pediatric use prior to initiation of clinical trials in human pediatric populations.

1155 AN ASSESSMENT OF THE USE OF DIMETHYL SULFOXIDE AS A SOLVENT FOR HYDROPHobic CHEMICALS IN A DROSOPHILA-BASED DEVELOPMENTAL TOXICITY PRESCREEN.
D. W. Lynch. Biomonitoring & Health Assessment Br, NIOSH, Cincinnati, OH.

Dimethyl sulfoxide (DMSO) is routinely used in toxicity testing to solubilize hydrophobic chemicals; however, the potential of DMSO to induce developmental toxicity in the Drosophila-based pre-screen has not been well characterized. The purpose of this study was to investigate this toxicity and to determine a maximum concentration of DMSO which can be used. Three separate experiments were conducted using DMSO (CAS# 67-68-5; Sigma D-5879, 99%) at concentrations ranging from 0.05 - 150 μg/ml; Drosophila were exposed throughout development (egg through third instar larva) in culture vials to medium containing DMSO. A mixed, untreated, Oregon-R wild-type female was added to each vial and allowed to oviposit for 20 hours, then removed. Emerging offspring were collected over 10 days, and examined microscopically (25x) for bent humeral bristles and wing blade notches; morphological defects shown to occur with an increased incidence in flies exposed to developmental toxicants. In each experiment, the incidence of the two defects at each concentration was compared to the concurrent controls using chi-square. Where replicate data were available at a given concentration, incidence data were also pooled and compared to the pooled controls. No flies emerged at concentrations greater than 50 μg/ml. In the first experiment the incidence of bent humeral bristles was significantly increased at 25 μg/ml, 64/246 (p = 0.001) and at 50 μg/ml, 2/2 (p = 0.001); in the second experiment bristle defects were only at 50 μg/ml, 5/6 (p = 0.001); in the third experiment bristle defects were increased at 1.0 μg/ml, 5/155 and 5.0 μg/ml, 5/146 (both p = 0.05). These results indicate that higher concentrations of DMSO can increase the incidence of bristle defects in developing flies. However, based on the lack of a statistically significant increase in defects at lower concentrations in the pooled data across 3 experiments, the data suggest that DMSO at a concentration of 10 μg/ml can be used to evaluate hydrophobic chemicals in this assay.

1156 MODIFIED DAVIDSON'S FIXATIVE AS A REPLACEMENT FOR BOUIN'S SOLUTION USED FOR FETAL EVALUATION UTILIZING WILSON'S TECHNIQUE.

Near-term rat fetuses have been traditionally placed in Bouin's solution for fixation and decalcification. This allowed adequate soft tissue evaluation following razor blade sectioning. While Bouin's solution produced good results, the contrast of the tissues could be improved. The picric acid component presents a health and safety hazard, and an environmental disposal problem. Davidson's fixative was modified at Huntingtonton Life Sciences by H. Joransen to be used as a replacement for Bouin's solution that is routinely used to fix reproductive organs, testes and epididymides. The modified Davidson fixative contains 14% of pure ethanol, 6.25% glacial acetic acid, 37.5% saturated formaldehyde and 42.25% distilled water. The fetuses are placed in a plastic jar containing modified Davidson's fixative for one week. After fixation, the fetuses are rinsed twice in tap water and stored in 70% Isopropyl Alcohol. The razor blade section technique described by Wilson is also used to perform vibrational microtomy. Superior contrast and enhanced definition of organs and vessels are achieved in the fetuses following fixation in Modified Davidson's fixative. Sections remained moist for an extended period of time during evaluation.

1157 INDUCTION OF STRESS PROTEINS BY ARSENIC COMPARED TO HYPERTERMIA IN TISSUES OF THE AVIAN EMBRYO. A. S. Schier and S. E. Bloom. Microbiology and Immunology, Cornell University, Ithaca, NY.

Exposure of the early embryo to environmental insults may result in different developmental outcomes depending on target dose, developmental stage, and tissue specificity. The induction of stress proteins may be important indicators of exposure and may influence developmental toxicity. The present study was designed to evaluate the early avian embryo's response to arsenic and hypertermia. We chose the 10-day-old chick embryo to study the induction of stress proteins by arsenic and hypertermia in target tissues. The study compared the induction of the heat shock proteins Hsp24, Hsp70, as well as c-jun and its phosphorlated form (P-c-jun) in the head and heart, which differ in stress resistance. K-strain chick embryos at 3 stages of development were exposed to graded dosages of sodium arsenite (AS) or to heat shock (HS) at 37°C for different periods to toxicity. Head and heart were harvested at 3, 6, and 10 hr post exposure to AS or HS. Then, stress protein induction was analyzed using immunoblotting analysis. Constructive levels of Hsp70 and c-jun were similar in the 2 tissues, but constitutive P-c-jun expression was 7-fold higher and Hsp24 was 3-fold higher in the heart. Embryos treated with AS doses of 125, 200, and 250 μg/g demonstrated a dose-dependent increase in Hsp24, Hsp70, and P-c-jun by 10 hr with consistently and substantially higher expression in the heart. C-jun expression in response to AS was similar in the heart and head. AS compared to HS treatments were also evaluated for their capacity to induce stress responses. In the heart, H5 induced P-c-jun and Hsp24 at a 50-fold differential over comparable AS doses at 3 hr. H570 induction by HS was also greater than AS by about 4-fold. Non-lethal HS treatments were effective at inducing Hsp24 and Hsp70, whereas comparable AS treatments failed to elicit significant responses in head and heart. Thus, profiles of differentially induced stress proteins may be useful as indicators of agent-specific exposures at the tissue level. Also, differential stress protein induction in the developing head and heart may contribute to regulating tissue sensitivity and overall developmental toxicity.


The FDA Modernization Act of 1997 and the final FDA rule on April 1, 2001 require companies to address the safety and effectiveness of drugs and biologicals in pediatric patients. One way to address the presumed higher risk in children is to conduct safety evaluations during critical developmental stages using non-ratinal animal models. However there is limited reference data available for non-ratinal species. This study presents reference data for male and female Beagle dogs for defined developmental stages: term neonate (<3 weeks), infant/toddler (3-6 weeks), child (6-20 weeks) and adolescent (20-28 weeks). These data are derived from 7 to 10 Beagle litters whelped at Will Research Laboratories, Inc., the Beagle pups received no treatment over the course of gestational/postnatal development. Mean values and standard deviations are presented for endpoints collected over the course of the study. Parameters evaluated include growth patterns (body weight, height, length and tarsus circumference); developmental landmarks (skin coloration, eye lid separation, balanopreputial separation, vaginal patency and oris descent); total body composition (lean vs non-lean tissue) and bone mineral density, mineral density and bone mineral content); locomotor activity assessment; and standard clinical chemistry parameters (serum chemistry and hematocrit) are all presented to demonstrate patterns of change over the duration of Beagle development. The results of this study indicate that over the course of development predictive patterns of physiological changes can be measured and that these patterns can be associated with the defined pediatric developmental stages. Reference data from the Beagle dog will be essential for the design of non-ratinal pediatric studies and in the...
terpretation of findings for addressing the potential of pediatric risks from direct product exposure in regulatory toxicology studies employing non-rodent animal models.

1159 AN EVALUATION OF THE POTENTIAL OF TWO PEPTIDES, PRAMLINTIDE AND AC2993 (SYNTHETIC EXENDIN-4), TO CROSS THE HUMAN PLACENTAL BARRIER USING AN IN VIVO PERFUSION SYSTEM.

R. A. Hiles, R. E. Bawdon and E. Petrela.

Pramlintide (a 37-amino acid peptide) and AC2993 (synthetic exendin-4; a 39-amino acid peptide) are under development for treating patients with diabetes mellitus. Because of the chronic nature of diabetes and, thus its treatment, some patients will become pregnant while on therapy. Therefore, it is important to have knowledge of the potential for direct exposure of the developing fetus to these exogenous peptides. The use of human placentas obtained from term pregnancies in an in vivo evaluation system allows this situation to be studied without the risk associated with an in vivo evaluation. The placental cotyledon selected for each perfusion (6 per peptide) met the acceptance criteria set by Chatlier. Insulin (added as a control peptide known to cross the placental barrier) and the study peptide were added to the maternal side of the perfuse and both the maternal and fetal sides were sampled over the 2-hour equilibration perfusion period and assayed for insulin and the study peptide. Measured insulin on the maternal side of the perfuse remained elevated (4-0, 000 μU/mL while the concentration on the fetal side remained low (<10 μU/mL). With mean pramlintide concentrations on the maternal side of 15, 000 to 43, 000 ng/mL the concentrations on the fetal side ranged from 28 to 268 ng/mL (fetal-to-maternal ratio of 0.002 to 0.037). These results suggest the potential for either pramlintide or AC2993 to cross the human placental barrier is very low. Chatlier JC. Criteria for evaluating perfusion experiments and presentation of results. Contr. Gynec. Obstet. 1985; 13:32-9.

1160 INCIDENCE OF VARIATIONS AND MALFORMATIONS DURING FETAL DEVELOPMENT IN THE CYNOBOLUS MONKEY.


The cynomologus monkey (Macaca fascicularis) is the predominant species for investigation of embryo-fetal development in the non-human. Given the current momentum of development of new biotechnology products and immunotherapeutics, it is obvious that the demand for developmental toxicity studies in this primate model will increase. In order to be able to distinguish between spontaneous and compound-induced developmental alterations, a comprehensive and reliable data base is mandatory. We therefore investigated 399 fetuses obtained at gestational day 100 and 63 fetuses obtained at gestational day 150 from control mothers. The parameter chosen for assessment was skeletal development. The ossified skeleton was stained with Alizarin red and the carcass was made translucent by preservation in glycerine. A highly graded evaluation scale for identifying even minimal variations was used. At day 100 minor variations were observed in nearly 90% of the fetuses, whereas malformations were encountered in seven fetuses only (1.7%). The most frequent variation was observed in the sternum followed by ribs, vertebral column and skull bones. At day 150 minor variations were also seen in the sternum and ribs at an incidence of 75%. However, malformations could not be detected. These data provide an excellent basis for the characterization of normal fetal development during developmental toxicity studies in the cynomologus monkey model.

1161 EVALUATION OF TRICHLOROETHYLENE IN A RAT INHALATION DEVELOPMENTAL TOXICITY STUDY.


As part of a voluntary program to fill "priority data needs" identified by the US Agency for Toxic Substances and Disease Registry (ATSDR), an inhalation developmental toxicity study conforming to current global regulatory guidelines was conducted. Groups of twenty-seven time-mated, female CD rats were whole-body exposed to target concentrations of 0, 50, 150, and 600 ppm vaporized trichloroethylene (TCE) for six hours/day, seven days/week on days 0-20 of gestation in order to evaluate the maternal and developmental toxicity potential of this compound. The level of 600 ppm represents a limit dose defined in EPA guidelines for inhalation studies. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain, and feed consumption. On day 21 of gestation, all rats were euthanized and examined for gross pathologic alterations. Liver, kidneys, and gravid uterine weights were recorded, along with the number of corpora lutea, implantations, resorptions, and live/dead fetuses. All fetuses were weighed, sexed, and examined for external alterations. Visceral or skeletal examinations, each involving approximately one half of the fetuses, were conducted as designated. Visceral examinations included visualization of the internal structures of the heart. In addition, the internal structures of the head were examined by serial sectioning for one half of the fetuses. Dams exposed to 600 ppm TCE exhibited maternal toxicity as evidenced by significant decreases in body weight gain (22.5% less than the control group) on gestation days 6 through 9. There were no maternal effects at either 50 ppm or 150 ppm TCE and no indications of developmental toxicity (including teratogenicity) at any exposure level. Therefore, the no-observed-effect-concentration (NOEC) for maternal toxicity was 150 ppm, whereas the embryofetal NOEC was 600 ppm. Funded by the Halogenated Solvents Industry Alliance, Inc.

1162 THE AFFECT OF FUMONISIN B1 ON FOLATE TRANSPORT IN CELLS DERIVED FROM MURINE EMBRYOS THAT ARE SUSCEPTIBLE TO FBL-INDUCED NEURAL TUBE DEFECTS.

S. Heller, L. Gelineau-vaes, J. R. Maddox, M. Solomon and G. D. Riggs. Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE.

Fumonisins are mycotoxins that are produced by Fusarium moniliforme (F. verticilloides) and are contaminants of grains, particularly corn. Their distribution is worldwide and there are at least 14 known fumonisins, of which fumonisin B1 is the most common. FBL is associated with fatal toxicosis in horses and pigs and it causes hematotoxicity in rodents. Several studies have linked FBL to epidermal cancer in humans. Of the few studies that have investigated the teratogenicity of FBL, most have found very little evidence that this mycotoxin causes any abnormalities other than a generalized embryotoxicity. However, recently we have demonstrated a strain difference to FBL-induced NTDs in two strains of mice, where the pregnant dams were acutely treated during the early stages of gestation. It has been demonstrated that FBL can disrupt the transport of folate into cultured cells. Additionally, folate deficiencies have been associated with NTDs in both humans and rodents. Therefore the current study was designed to determine whether FBL could alter the transport of folate into cultured primary embryonic cells derived from both susceptible and resistant strains of mice. Once established, primary cells were cultured for 2 days in folate free media during which time a group of cells were also exposed to 20 μg/mL of FBL. At the end of this culture period folate uptake was assayed, using [3H]-folate, from 15 to 60 minutes. Preliminary results from these studies demonstrate that FBL exposure lead to a decrease in folate uptake in the cells from the susceptible strain. In contrast, the cells from the resistant strain were unaffected by FBL. These differences in folate uptake may lead to a defect in folate transport in susceptible embryos and may explain the teratogenic differences seen between these two strains of mice.

1163 EX VIVO HUMAN PLACENTAL PERFUSION SYSTEM IN THE ASSESSMENT OF TRANSPLACENTAL PASSAGE.

P. K. Millipenn, P. Pienimaki and K. H. Vahakanagi. Department of Pharmacology and Toxicology, University of Oulu, Oulu, Finland and Department of Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland. Sponsor: K. Savolainen.

Ethical considerations limit in vivo human studies of transplacental passage of drugs. Animal testing gives valuable information but the structure and function of the placenta are species specific. Our aim is to clarify the usefulness of placental perfusion methods as a part of pre-clinical risk assessment. Single human placental cotyledons were perfused for 2 hours. Modified Krebs-Ringer phosphate buffer with natriumcarbonate, dextar and buparin was used as perfusion medium. In successful perfusions the leak from maternal to fetal side was less than 2 ml/h. Carbamazepine (5-carbamoyl-5H-dibenzo[b,f]azepine, CBZ), oxazepam (10, 11-dihydro-10-oxo-5H-dibenzo[b,f]azepine), diazepam (5-carboxamide), OCZ2) and lomac- migine (3, 5-diamino-6-2, 4-dichlorophenyl)-1, 2, 4-triazine, LTG) were studied. Antipyrine, a non-iodized, non-metabolized and non-protein bound drug, was used as a reference substance. Drug concentrations were analyzed using high performance liquid chromatography. In addition, cord blood and maternal venous samples were collected from mothers using OCZ2, CBZ and LTG during pregnancy. All studied substances crossed placenta rapidly, maternal and venous concentrations being equal at steady state. Equilibrium between maternal and fetal concentrations was reached earlier with CBZ, OCZ2 and LTG than with antipyrine.
Although data achieved with placental perfusion method usually correlated well with available clinical data differences between in vivo and ex vivo situation were also noted. Perfusions indicated slight accumulation of 10-OH-CB2 in placental tissue but in vivo results indicated no accumulation. This may have been due to tissue oedema during perfusions. In conclusion, human placental perfusion system is a promising method but further studies are needed to validate the method for the assessment of fetal exposure.

**1164** INTERLABORATORY STUDY OF THE PRIMARY ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS IN OUTBRED RODENTS.

S. E. Lovelace1, G. S. Fadiga1, C. Smith1, M. P. Holdgate2, M. R. Woolhouse2, P. K. Anderson1, K. L. White Jr.1, D. L. Musgrove1, R. J. Smirnoff2, and W. Williams2. 1DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, DE; 2Dow Chemical Co, Midland, MI; 3Virginia Commonwealth University, Richmond, VA; and 4NIEHS, Research Triangle Park, NC.

EPA guidelines currently provide a choice in evaluating immune system function in rats and mice using sheep red blood cells (sRBC) as the preferred antigen, namely, an antibody-forming cell (AFC) assay or a sRBC-specific serum IGM ELISA. The initial objectives of this interlaboratory study were for all four laboratories to use the same source of sRBC and determine (1) optimal sRBC concentration for immunization and (2) peak day of antibody response in female CD rats and CD1 mice. The primary objective was for each Lab. to determine which of the two methods was more sensitive in detecting suppression of the antibody response by cyclophosphamide (CP). Each Lab. determined optimal immunization concentrations for each assay and each species. Peak antibody response was day 4 for the AFC in mice or rats, whereas Day 5 was the peak day for the ELISA assay in mice and Day 6 for rats (sRBC injection was on Day 0). Rats were injected with sRBC followed by ip injections of 0, 0.3, 1, 3, 10, or 30 mg/kg/day CP. Statistically significant reductions in antibody response were first detected at 1 mg/kg/day (44% suppression, AFC/million splenocytes) and at 10 mg/kg (36-69% suppression by ELISA by 3 labs). Mice were injected with sRBC followed by ip injections of 0, 5, 10, 20, 40, or 60 mg/kg/day CP. Statistically significant reductions in the antibody response were detected at 5 mg/kg (36-64% suppression - AFC assay) by 3 labs and 10 mg/kg (55% suppression) by the remaining Lab. Three labs detected suppression at 10 mg/kg (50-25% inhibition) using the ELISA. In conclusion, the rat ELISA detected significant suppression at the same (2 labs) or lower (1 Lab.) concentration compared to the AFC assay. In contrast, the mouse AFC assay detected suppression at a lower dose than the ELISA in 3 of 4 labs.

**1165** THE RESPONSE OF C57BL/6 MICE TO THE MOUSE INFLUENZA HOST RESISTANCE (MIHR) MODEL.


In safety testing of potential immune modulators, one of the major objectives is to determine if the compound has an immunosuppressive effect, resulting in an increased susceptibility to infectious disease. Since clearance of the influenza virus requires an intact functional immune system, incorporating cytokine production, antibody production and natural killer cell, macrophage, and cytotoxic T lymphocyte activity, the MIHR model allows an assessment of overall immunocompetence. Although the MIHR model is well characterized in BALB/c mice, there has been limited experience with this model in C57BL/6 mice. The purpose of this study was to characterize the response of the C57BL/6 mouse strain in the MIHR model, in order to establish baseline parameters that could be used to assess immune competence in specific knock-out mice on the C57BL/6 background. Male and female C57BL/6 mice, 6-8 weeks old were administered influenza virus (A/PR/8/34) by ic (4 X 105 (lo lnV) or 4 X 104 (hi lnV) infectious particles), with or without daily oral dexamethasone (DEX) (Day 3 through Day 6 or Day 21) as a positive control. Viral burden in the lung homogenate and InV-specific IgG titers were determined at several time points postinfection. The 10 day survival rate in the C57BL6 hi lnV was 56.0% in males with 10 mg/kg/d DEX, 96.7% in male vehicle controls, 73.3% in females receiving 10 mg/kg/d DEX, and 96.7% in female vehicle controls. In a second study with female mice given 5 mg/kg/d DEX, there were 72% survivors in the hi lnV group and 96% survivors in the lo lnV group. With 1 mg/kg/d DEX, with hi lnV, there were 96% survivors; vehicle control survival was 92.100%. Histological control BALB/c female mice receiving 20 mg/kg/d lo lnV or vehicle, had 100% 10 day survival. This study demonstrated in comparison to BALB/c mice, the C57BL/6 strain is more susceptible to InV and DEX in the MIHR, and reinforces that establishing strain-specific characterization of the MIHR is critical for accurate assessment of an immunotoxic potential.

**1166** COMPARATIVE ANALYSIS OF HUMAN, CYNOGOLUS MONKEY AND COMMON MARMOSET PERIPHERAL BLOOD MONONUCLEAR CELLS BY FLOW CYTOMETRY.


Flow cytometry has become a recognized method to measure mononuclear cell numbers on samples taken from toxicology studies. For reasons of epitope expression and biopharmaceutical immunogenicity, non-human primates are often the toxicological species of choice. In the absence of reagents specific for non-human primates, commercially available enzymes for human monoclonal antibodies were evaluated in cynomolgus monkeys and marmosets, using multiple colour analysis to improve the accuracy of immunophenotyping and reduce blood requirements. EDTA-blood was stained with cocktails of monoclonal antibodies the human and cynomolgus samples lysed with TQ Prep (Beckman Coulter) or Phamlyse (BD Pharmingen) for the marmoset samples. The non-human primate samples were analysed for total T lymphocyte (CD3+)+ and B lymphocyte (CD20+)

**1167** HUMORAL IMMUNE RESPONSE TO KEYHOLE LIMPET HAEMOCYANIN (KLH) IN THE CYNOGOLUS MONKEY.


The safety assessment of immunomodulatory biopharmaceuticals require immunopharmacology/toxicity studies to be performed in a pharmacologically active species. Because species selection may have to take account of restricted epitope expression, ligand specificity and immunogenicity of human proteins, the non-human primate may be the most relevant species. Accordingly, the humoral immune response to the T-dependent antigen KLH was performed in the cynomolgus monkey to monitor specific antibody production. Groups of cynomolgus monkeys were immunized with KLH using 3 different protocols, then specific antibody to KLH was measured using a capture ELISA. In protocol 1, the animals received 2x100ug of KLH i.d. 43 days apart. Primary and secondary antibody responses were observed in all animals, however the variability in the response suggested that the dose of antigen or its formulation were not optimal. In protocol 2, the time between the two immunizations was reduced to 14 days to fit the procedure into a 4-week regulatory toxicity study. The animals received 2x100ug of KLH i.d. with the first dose formulated in Freund's incomplete adjuvant. This resulted in a robust and homogeneous antibody response, however the primary and secondary antibody responses were merged. In protocol 3 the animals received 2x100ug KLH s.c. 14 days apart. The antibody response was homogeneous and robust, plus the primary response which typically plateaued by day 15, was distinguishable from the secondary response. In conclusion, protocol 3 offered the advantage of producing a homogeneous response without the need to use adjuvant and importantly allowed the primary and secondary responses to be monitored within a 4-week regulatory toxicity study design.

**1168** ANALYSIS OF RAT IMMUNOTOXICITY, HEMATOLOGY AND CLINICAL CHEMISTRY PARAMETER VARIABILITY.

G. Healey, D. J. Lanham and M. G. Wing, Experimental Biology, Huntington Life Sciences, Huntington, United Kingdom. Sponsor: C. Atterwill.

Common methodologies are cited in regulatory guidelines for the immunotoxicity testing of pesticides, industrial chemicals, food additives and NCEs. These include immunophenotyping by flow cytometry, natural killer (NK) cell activity in a chromium release assay and specific antibody formation in a plaque forming cell (PFC) assay. Concerns have been raised over the variability seen in these assays and so immunotoxicology data were compared with clinical chemistry, haematology and organ weight data from a series of rat studies. Using data from control or unal-
1169
IDENTIFICATION OF THE AGE AT WHICH IMMUNOTOXICOLOGICAL PARAMETERS CAN BE EVALUATED IN RATS.
R. A. Marulka1 and R. J. Siminowicz2, 1Curriculum in Toxicology, UNC, Chapel Hill, NC and 2ITB, ETD, USEPA, Research Triangle Park, NC.

Development of the immune system begins early in gestation and continues to mature throughout puberty. Exposure to immunotoxic agents may occur at any point in development (i.e., prenatal, early post-natal, juvenile). However, the immune function assays have been traditionally performed in adult rats, however, these assays have not been adequately tested for application during early post-natal and/or juvenile immune system development. The objective of this study was to determine the age at which immunotoxicological assays on juvenile Sprague-Dawley rats. Animals were tested for contact hypersensitivity (CHS), delayed type hypersensitivity (DTH) responses to bovine serum albumin (BSA), primary immune response, as demonstrated by increases in anti-scrub red blood cell (SRBC)-specific IgM. Sensitization or immunization was initiated at 3, 4, 5, or 6 weeks of age. At 3 weeks of age both males and females demonstrated elevated CHS responses at DNP, (i.e., 52.8% and 53.1% increase over naive controls, respectively). In contrast, DTH response was absent in males, and only minimally increased in females. Anti-SRBC IgM production was determined as ELISA, averaged 4.3 and 7.9 μg/mL for males and females (adult concentrations average 30 μg/mL). At 4 weeks of age, the CHS response was 14% and 7% over control for females and males, respectively. DNP production averaged 15.3 μg/mL in males, and 18 μg/mL in females. DTH response showed a 16% and 14% increase in males and females, respectively, and the CHS response in males and females increased 62% and 36%, respectively. These results indicate while the immune function response employed produced measurable reactions in these three experimental procedures, the protocols must be refined for testing juvenile animals. (This abstract does not reflect EPA policy.)

1170
VALIDATION OF A METHOD TO DETERMINE RELATIVE PROPORTIONS OF ERYTHROID, MYELOID AND LYMPHOPRECURSORS IN RAT BONE MARROW BY FLOW CYTOMETRY.

To assess toxicity in the bone marrow, we have validated an assay to determine the proportions of myeloid, lymphoid and erythroid cells in rats using flow cytometry based on the method described by A. Saed et al. “Differential Analysis of Rat Bone Marrow by Flow Cytometry,” Comparative Haematology International (2000) 10:97-101. Bone marrow cells from rat femurs were stained with fluorochrome conjugated antibodies against CD71 and CD45. Cells were acquired using a flow cytometer and gated according to their expression of CD71 and CD45. Cells with a high level of CD71 expression were regarded as erythroid. A gate was placed around the remaining myeloid and lymphoid cells and these two populations were distinguished according to their side scatter properties. Cell proportions were calculated as a percentage of the total bone marrow population. Parameters examined in this validation were intra- and inter-assay precision, reference range and stability of fixed samples. Results for both the intra- and inter-assay precision for myeloid/lymphoid cells were within 10% of J200. Reference range results showed the proportion of erythroid cells ranged from 31.27 to 38.33% in males and 32.88 to 43.21% in females. Myeloid/lymphoid cell proportions varied between 32.85 and 42.30% for males and 27.67 to 41.31% for females. When a gate was set only on the myeloid/lymphoid population, the proportion of myeloid cells represented 45.36 to 53.88% in males and 44.13 to 52.23% in females. The proportion of lymphoid cells ranged from 40.42 to 50.93% for males and 44.21 and 50.21% in females. Results for stability were rejected as the isotype controls at 48 and 72 hours displayed a high level of fluorescence. Intra- and inter-assay precision met the acceptance criteria and the reference range for males and females was determined. Stability was not acceptable at 48 and 72 hours, therefore samples must be analyzed within 24 hours of staining.

1171
FURTHER INTEGRATION OF THE MOUSE LOCAL LYMPH NODE ASSAY.
M. R. Woolliiser, P. A. Anderson and M. P. Hottappel, Toxicology & Environmental Research and Consulting, The Four Chemical Company, Midland, MI.

The LLNA has been extensively characterized and is now accepted as a “stand alone” to the traditional guinea pig model to assess the sensitization potential of chemicals. The goal of this investigation was to facilitate the continued integration of the LLNA. First, we have begun using an approach whereby a test material (TM) is evaluated for primary irritation in a pre-screen, range finding study. Briefly, at least six concentrations of TM are applied to the ears of mice for two days and ear swelling is determined 24 hrs later. Based upon measurable, detectable elevations in ear thickness, and reported increases in ear swelling for known irritants, we identify the concentration that produces 10% ear swelling as the minimal irritating concentration (MIC). The concentrations used in the subsequent LLNA are based on the MIC. The validity of this method is demonstrated with benzalkonium chloride (BC) and known irritants and sensitizers. Administration of the MIC for non-sensitizers produces a stimulation index (SI) near or less than 3.0, the standard threshold for a positive LLNA. Second, the standard LLNA protocol calls for a 5-hour pulse with H-chymidine. This amount of time is not consistent with what is known about cellular proliferation or the uptake of radiolabel. A reduction in pulse time would reduce the time of the study and introduce flexibility in the design. Initial studies with strong sensitizers (5% TMA and 1% DNCB) indicated no significant differences in absolute dpms after a 2-, 3- or 5-hour pulse. However, the absolute dpms in the vehicle groups were decreased at the earlier timepoints; this ultimately had an impact on SI values. This trend repeated when BC or the mild sensitizers, HCA, were evaluated and resulted in a decrease in SI that were slightly greater at 2 hours than at 5-hours despite similar dpms values following TM exposure. These data indicate that a shorter pulse is possible, thus providing flexibility in the conduct of the LLNA. These studies also highlight the importance of using both SI and absolute dpms for the importance of the LLNA.

1172
TRITIATED THYMIDINE INCORPORATION AS AN ENDPOINT IN THE POPULATED LYMPH NODE ASSAY.
C. Ruan1, L. Faure2, G. Chocquet-Kasyeliski3 and J. Descotes2, 1IMDS Pharma Services, Laurière, France and 2Union Center & INSERM University of S03, Edouard Herriot Hospital, Lyon, France.

The poplitel lymph node (PLN) assay is an investigative tool to predict drugs and chemicals that are likely to induce systemic autoimmune and immunogenic reactions in man. Typically, the endpoints used are lymph node weight and cellularity, but sensitivity is not considered optimal. In the present study, incorporation of tritiated thymidine was used. Groups of 8 Balb/c mice received 50 μl of 10, 20 or 40 mg/mL of streptozocin, diphenylylhydantoin, sulfamethoxazole, or meformin into the hind footpad. Two additional groups received either 20 mg/mL of imipramine or 50% ethanol. The same volume of the vehicle was injected into the other hind footpad. Seven days later they were sacrificed five hours after an intravenous injection of 20 μCi of tritiated methyl-thymidine. PLNs were weighed and the incorporation of tritiated thymidine was measured by liquid scintillation counting. PLN weight and thymidine incorporation were increased to a similar degree relative to the control limb with streptozocin, diphenylylhydantoin, imipramine and ethanol. No change was seen in either endpoint with sulfamethoxazole and metformin. Based on these results, the measurement of thymidine incorporation does not seem to enhance the sensitivity of the assay. In addition, it failed to identify either imipramine or ethanol as inducing a non-specific inflammatory response. Finally, the negative results obtained with sulfamethoxazole indicates that this assay will not readily detect immunogenic compounds.

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FURTHER EXAMINATION OF AN ALTERNATIVE VEHICLE FOR WATER SOLUBLE MATERIALS IN THE LOCAL LYMPH NODE ASSAY.
L. W. Greve1, C. A. Ruan1, R. A. Skinner1, R. J. Deanarm1, L. Kimber1 and G. E. Getbeck1, 1The Procter & Gamble Company, Cincinnati, OH and 2Syngenta CTT, Macclesfield, United Kingdom.

Vehicle choice for the murine local lymph node assay (LLNA) is important and has been shown to influence the sensitization potential of hapten. Suggested vehicles include organic solvents and organic-aqueous mixtures. However, due to its poor
wetting qualities and high surface tension, water is not recommended as testing water soluble materials can be problematic. We previously identified a water-based vehicle that provided better skin wetting properties than water alone and assessed its performance in the LLNA relative to other solvents using two water-soluble reactants, diisobutyleneamine and formaldehyde. This wetting vehicle, 1% aqueous Fluoronic L92 (0.92) has been further examined using the metal salts potassium dichromate and nickel sulfate and its performance compared to di-(2-(2-methoxyethoxy)ethoxy)ethanol (DMSO) and dimethylsulfoxide (DMSO). Estimates of the relative sensitizing potency in each vehicle were determined by calculation of EC3 values (the estimated concentration required to induce a threshold positive response). Potassium dichromate was positive in DMSO, DMSO and 1% L92. The potency ranking was DMSO > DMSO > 1% L92 (EC3 values were 0.03%, 0.05% and 0.17%, respectively). Potassium dichromate in water was negative. Nickel sulfate induced threshold positive responses when formulated in DMSO and the test vehicle. The test vehicle (1% L92, stimulation indices of 3.1 and 3.6, respectively) whereas the maximal stimulation index recorded following application in DMSO was 2.2. Based on the results of this study, we recommend either DMSO or 1% L92 as the preferred vehicle for water soluble materials in the LLNA. However, if the chemical is not soluble in DMSO or 1% L92, the test vehicle (1% L92) provides a better alternative to water alone in terms of improved assay performance for hazard identification.

**USE OF A B CELL MARKER (B220) TO DISCRIMINATE BETWEEN ALLERGENS AND IRITANTS IN THE LOCAL LYMPH NODE ASSAY (LLNA).**

G. E. Gerberich,1 L. W. Cruse,2 C. A. Ryan,1 J. G. Chaney,1 R. A. Skinner,3 R. J. Dearman4 and L. Kimber1,5 The Procter & Gamble Company, Cincinnati, OH and Syngenta CTL, Macclesfield, United Kingdom.

It has been shown that mice exposed to contact allergens show selective B cell activation in the draining lymph nodes (DLN) as seen by an increase in the % of B220+ or IgG1+ B cells. We now examine whether depletion of B220+ B cells could be used as an alternative endpoint for the LLNA to differentiate between allergenic responses and those few irritants which induce low level proliferation in the DLN. Mice were treated on the ears for 3 consecutive days with concentrations of allergens or irritants known to cause proliferation in DLN cells. The DLN were excised 72 h after the final treatment and the cells were prepared for B220 analysis by flow cytometry. The % B220+ cells from test and vehicle treated mice were determined for 5 allergens and 4 irritants in multiple experiments (n=3 to 17). As expected, the % of B220+ cells was increased with the allergens tested, whereas the % B220+ cells was not increased with irritant treatment. The % B220+ values for each chemical treatment (41 allergens and 28 irritant observations) versus the % B220+ values in the matched vehicle control were plotted and a classification tree model was developed to calculate a B220 test/vehicle ratio cutoff of 1.25 for discriminating between allergens and irritants. Using this ratio, 93% of the allergens and irritants were classified correctly. Moreover, the method was very reproducible, with the strong allergen 1-chloro-4,4-diisobutylazo-stilbene classified correctly in 17 of 17 experiments and the weak allergen 3-phenyl-4-hydroxyphenylacetamide classified correctly in 12 of 13 experiments. To evaluate the performance of this model in a second independent laboratory, 3 allergens and 2 irritants were tested. Each of the allergens and irritants were classified correctly using a B220 test/vehicle cutoff of 1.25. These data demonstrate that analysis of B220 expression on B cells may be useful in differentiating allergen and irritant responses in the DLN of chemically treated mice.

**PRE-VALIDATION STUDY FOR THE POPULTEAL LYMPH NODE ASSAY AS A TOOL TO PREDICT THE IMMUNOSENSITIZING CAPACITY OF (NON-) PHARMACEUTICALS.**


A large number of pharmaceutical drugs and non-pharmaceutical chemicals are known to cause systemic hypersensitivity and autoimmune-like clinical phenomena. The multifactorial pathogenesis of chemical-induced hypersensitivity is a major problem in developing models that allow recognition of the immunosensitizing potential of chemicals. To date no validated and generally applicable predictive immunotoxicological screening methods are included in traditional toxicity testing. The reporter antigen- popliteal lymph node assay (RA-PLNA) is regarded as a promising tool for this purpose as the RA-PLNA in particular is able to discriminate between compounds acting as adjuvant without stimulating specific T cell responses and chemicals that stimulate neo-antigen specific T cells. So far this system has not been validated. We studied specific immune responses in the RA-PLNA and compared those with responses in the auricular lymph node (ALN) after s.c. injection of the drug together with the RA TNF-Ficol into the ear (importantly s.c. injection between the ears did not elicit a TNF-specific response in the ALN).

Responses showed similar proliferation of Th, Tc and B cells in the assays but the increase in dendritic cells was higher in the RA-PLNA. TNF-specific antibody responses were also more increased in the PLN than in the ALN. Clearly, results re-veal that both assays, RA-PLNA and "RA-LLNA" are capable of identifying sensitising chemicals (DNP-penciclovin, estradiol, streptomycin, olsalazine, but that the RA-PLNA is more sensitive. Additionally, the narrow test system requires injection of smaller volumes thus limiting the amount of chemical that can be injected. This also causes difficulties with injection of non- or poorly soluble chemicals and evidently affects reproducibility. Together, this study contributes to the validation of the RA-PLNA as a predictive test for immunosensitizing capacity of chemicals and indicates the advantage of footpad injection over ear injection.

**PREDICTION OF HUMAN SENSITISATION POTENCY USING LOCAL LYMPH NODE ASSAY EC3 VALUES.**

D. A. Basketski,1 Z. Wright1, N. J. Gilmour,1 C. A. Ryan,2 G. E. Gerberich,2 M. R. Robinson1, R. J. Dearman1 and L. Kimber1,5 SEAC, Unilever Colworth Laboratory, Sharnbrook, United Kingdom, 1Syngenta CTL, Macclesfield, United Kingdom, 2Procter & Gamble, Miami Valley Laboratories, Cincinnati, OH and 5Depts of Health, London, United Kingdom.

The local lymph node assay (LLNA) is an alternative to guinea pig tests for identification of skin sensitising chemicals. In the standard LLNA protocol, CBA strain mice receive 3 consecutive daily topical applications of test substance (at 3 dose levels) 5 days after the initiation of exposure, proliferative responses in lymph nodes draining the application site are measured by radioisotope thymidine incorporation in situ. Chemicals which induce proliferative responses in the mouse are considered to be skin sensitizers.

The assay has been endorsed as a stand alone method for the determination of contact allergic hazard by the FDA, the EPA and others following approval by the International Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The LLNA has also received formal acceptance by the European Centre for the Validation of Alternative Methods (ECVAM). Following these formal validations, the UK submitted the LLNA as an alternative method to the Organization for Economic Co-operation and Development (OECD). The application has now moved towards the Joint Meeting for final approval. Discussions are now underway concerning how to incorporate the LLNA into regulatory tests within the EU and within the Globally Harmonised System of toxicological testing.

**THE LOCAL LYMPH NODE ASSAY: CURRENT REGULATORY STATUS.**

Z. Wright,1 D. A. Basketski,1 R. J. Dearman1, C. A. Ryan2, G. E. Gerberich1, R. J. Fielder2 and L. Kimber1,5 SEAC, Unilever Colworth Laboratory, Sharnbrook, United Kingdom, 1Syngenta CTL, Macclesfield, United Kingdom, 2Procter & Gamble, Miami Valley Laboratories, Cincinnati, OH and 5Depts of Health, London, United Kingdom.

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1178 RESTRANT STRESS MODULATION OF EAR SWELLING IN ACD IS INDEPENDENT OF SENSITIZING DOSE OF CHEMICAL.

M. S. Flint, R. R. Salmont, K. Brunsmann and S. S. Tinkle. TMBB, NIOSH, Morgantown, WV.

Stress plays an important role in numerous chronic health problems including modulation of immunologic diseases, however, the interaction between the stress response and the cutaneous immune response is incompletely understood. We have previously shown that acute stress suppresses the immune response in allergic contact dermatitis (ACD), and additional studies have shown a significant interaction between the concentration of chemical required for sensitization and for challenge in the development of ACD. We hypothesized that 2 hour restraint stress would change the sensitizing concentration of chemical required to elicit a full ear swelling response to chemical challenge. Male BALB/c mice were exposed on the back with increasing doses (0.5%-5%) of 2,4-dinitrofluorobenzene (DNFB) on days 1 & 2 and challenged on day 3 with 0.1% DNFB on day 6. Mice were restrained for 2 hours prior to chemical application on day 1. We assessed ear swelling 24, 48, and 72 hours post-challenge. Quantitative analysis of the data indicated that the ear swelling response to chemical challenge is dependent upon the sensitization concentration of DNFB and is dose-responsive. The curve shows a quadratic trend with 0.1% as a possible maximum dose for sensitization. Application of restraint stress did not alter the shape of this curve but significantly suppressed ear swelling at all concentrations of DNFB. These data suggest that restraint modulation of ACD is independent of the sensitizing dose of chemical.

1179 EXPRESSION OF TH2 CYTOKINES IS A GENERAL FEATURE OF MURINE CONTACT ALLERGY.


The cytokine response pattern following sensitisation (induction) and elicitation (challenge) of contact allergy in BALB/c mice with different chemicals (dinitrochlorobenzene, dinitrofluorobenzene, oxazolone, glutaraldehyde, formaldehyde, trimellitic anhydride, croton oil) was investigated. The results of our investigations showed that contact allergens induced both Th1 helper (Th1) and Th2 cytokines interleukin (IL) 2, interferon-gamma (IFN-gamma) and IL-4, IL-5, IL-6 and IL-10 at different stages during murine contact allergy. We also confirmed our previous findings that IL-4 and IL-10 release were up-regulated during the challenge phase regardless of the contact allergen used, whereas the release of IFN-g did not show a clear preference for being up- or down-regulated. In our hands, the increased expression of Th2 cytokines after challenge exposure to contact allergens appeared as a stable marker of secondary contact allergic responses. Quantitative differences in the expression of IL-4 were observed between different contact allergens. The present results clearly indicate that skin sensitizers are able to elicit cytokine response patterns, which could not be related to a clear-cut Th1 or Th2 type of cytokine response. Furthermore, topical application of contact allergens produced different kinetics of cytokine secretion upon induction and challenge. In our hands, the co-expression of Th1 and Th2 type cytokines appeared as a universal consequence of dermal application of contact allergens to responsive mice. Our results also indicate that chemicals differ in their potency to induce the expression of these cytokines. Furthermore, the results do not support the hypothesis that changes in cytokine profile are due to a mutually exclusive manner depending on their preference to induce either contact or respiratory allergy. The results are expected to renew the discussion about the usefulness of the Th1/Th2 paradigm in certain areas of immunotoxicology.

1180 THE USE OF CD54 AND CD86 EXPRESSION ON THE HUMAN THP-1 AND KG-1 CELL LINES TO PREDICT CONTACT SENSITIZERS.


In regard to in vitro skin sensitization tests, dendritic cells (DCs) derived from human peripheral blood have been considered in the development of new tests. However, there are some problems with the use of human DCs in skin sensitization tests. These include availability of human blood, donor to vary donor and other problems. In the present study, we evaluated the use of two new human cell lines, THP-1 (monocytic leukemia cell line) and KG-1 (acute myelogenous leukemia cell line) in developing a method to identify contact sensitizers. naïve cells were used in our studies after comparing the usefulness of cytokine-derived cells and naïve cells. The expression of CD54 and CD86 on THP-1 and KG-1 cells was measured in normal individuals and non-allergens (e.g., SL5, methylic salicylate, DMSO). We found that only the allergens enhanced the expression of CD54 and/or CD86 significantly in a dose dependent manner. In particular, metal salts such as NiSO4 and CoSO4 increased only the CD54 expression. Additionally, we examined the signaling pathways of DCNB in order to study the intracellular mechanisms involved in the activation of THP-1 by DCNB. Results obtained using Western blotting showed that the phosphorylation of p38 MAPK was increased after 30-minute exposure to DCNB. Furthermore, SB203580, a specific inhibitor of the p38 MAPK, inhibited the up-regulation of CD54 and CD86 upon stimulation with DCNB. These results suggested that the up-regulation of CD54 and CD86 was coupled to the phosphorylation of p38 MAPK. This hypothesis was further evaluated using more than 20 of our materials. The expression of CD54 and CD86 on THP-1 and KG-1 cells used for predicting the sensitization potential of these materials was compared to guinea pig test results. A higher accuracy was observed with THP-1 (>70%) than with KG-1 cells. These results suggest that in vitro sensitization screening using these cell lines, especially THP-1 cells, are useful to predict various contact sensitizers.

1181 INTEGRATION OF THE PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) ASSAY INTO STUDIES TO IDENTIFY POTENTIAL CHEMICAL ALLERGENS.

J. A. Hochkiss, N. J. Woodhive and M. P. Holappa. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.

Establishing a predictive test to identify chemicals that may induce respiratory sensitization is a pressing need in safety assessment. Concerns over the introduction of novel protein allergens has renewed an interest in the PCA assay to measure anti-gen-specific IgE. This study compared the ability of the PCA assay to identify known protein (ovalbumin; OVA) and chemical (trimellitic anhydride; TMA) respiratory sensitizers. BALB/c mice were systemically sensitized to OVA using two i.p. injections seven days apart with 5% OVA in PBS (or PBS alone control). Two injections were collected 21 and 28 days following initial exposure. Serial dilutions of test sera were injected i.d. into the ears of BAlb/c mice or the shaved backs of Sprague-Dawley (SD) rats. OVA and Evans blue dye were injected i.v. 48 hours prior to the mice. The lowest dilution producing a positive reaction (2 × 2 × 2 mm spot) was recorded. Both homologous and homologous PCA assays indicated that Day 28 OVA serum had the highest titer. For the chemical allergens, A/J mice were treated topically with 25% TMA, 1% dinitrochlorobenzene (DNCB, dermal sensitizer) or ace-tonecholine (AChO, vehicle) on their shaved backs. Seven days later, 12.5% TMA, 0.5% DNCB, or OAA was topically applied to the ears. Serial dilutions of test sera, collected 7 days later, were injected into the ears of A/J mice or the shaved backs of SD rats. Four hours later, 12.5% TMA or 0.5% DNCB was topically applied at the injection sites and 0.5% Evans Blue dye was injected i.v. Only mice injected with TMA sera and challenged with TMA displayed a positive PCA reaction. ELISA confirmed the presence of a modest increase of total IgE in the DNCB serum, a marked increase of total IgE in OVA serum and no elevation of IgE in AChO serum. The timing of serum injection, test chemical application, and assay endpoint must be optimized. These data suggest that the PCA assay may be used to identify elevations in specific IgE induced by chemicals which may induce respiratory sensitization.

1182 CYTOKINE PROFILING FOR CHEMICAL SENSITIZERS: EFFECT OF DOSE.

R. J. Smialowicz1, L. M. Plintick2, S. E. Leveille3, G. S. Ladics4, M. P. Holappa5, D. M. Salitied6, and M. J. Selgrade7.1 NIEHS, USEPA, Research Triangle Park, NC. 2 Curr. in Toxico., Inc., Chapel Hill, NC. 3 Haskell Laboratory, DuPont Co., Newark, DE. 4 Dow Chemical, Midland, MI.

Chemicals encountered in both domestic and occupational settings may result in airway hypersensitivity (AHS). Because cytokine profiles generated during a T cell response are indicative of AHS (Th2) or contact hypersensitivity (CHS) (Th1), cytokine production is of interest in determining mechanisms by which chemicals could identify chemicals with AHS potential. In this study, female BALB/c mice were sensitized twice on the flanks and challenged 3 times on the ears using the airway sensitization trimellitic anhydride (TMA), or the contact sensitizer dimethochrome (DNCB). Doses were varied during either sensitization, challenge or both simultaneously. At various times following challenge, total mRNA was isolated from draining LN and analyzed by ribonuclease protection assay (RPA). When dose varied during sensitization only, TMA induced higher levels of the Th2 cytokines IL4, IL10 and IL13 than DNCB. However, no dose-dependent responses were observed with either chemical. DNCB did not induce Th1 cytokines at any dose tested. Variation of TMA dose during both sensitization and challenge also induced IL4, IL10 and IL13, and again showed no dose dependency. Dose only appeared to be a factor when TMA concentration was varied during challenge alone, suggesting the magnitude of the response depends upon the challenge phase. Thus, dose appears to affect quantitatively differences in Th2 responses between TMA and DNCB. These results suggest conventional cytokine profiling studies in which the same dose is
1183 CYTOKINE PROFILING FOR CHEMICAL SENSITIZERS USING THE RIBONUCLEASE PROTECTION ASSAY: DETERMINATION OF CYTOKINES GENERATED BY ISOCYANATES.

L. M. Pintnick, S. E. Lorenz, G. S. Ladics, M. P. Holmapple, M. J. Selgrade, D. M. Sailor, and R. J. Smialowicz. *Curriculum in Toxicology, UNC, Chapel Hill, NC; *Haswell Laboratory, DuPont Co., Newark, DE; *Dow Chemical, Midland, MI; and *NIH/ERL, USEPA, Research Triangle Park, NC.

Certain isocyanates and acid anhydrides, which have been associated with occupational asthma, appear on the Hazardous Air Pollutant list (Clean Air Act 1990). Recent reports suggest that respiratory sensitizers may be identified based on their ability to induce a cytokine profile characteristic of a Th2 T-cell response. Previous studies from this laboratory have shown that RNA message levels for the Th2 T-cell cytokines IL4, IL10, and IL13 are elevated in response to the respiratory sensitizers trimellitic anhydride (TMA) and these increases may be detected by ribonuclease protection assay (RPA). In the current study, 4 additional chemicals (toluene diisocyanate (TDI), diphenylmethane-4,4′-diisocyanate (MDI), dicyclohexylmethane-4,4′-diisocyanate (HMDI) and isophorone diisocyanate (IPDI)) have been evaluated for their ability to induce Th2 cytokine mRNA expression. TDI and MDI are known respiratory sensitizers; however, there is conflicting data in the literature concerning the respiratory sensitizing potential of HMDI and IPDI. In the studies outlined here, female BALB/c mice were topicaly exposed to each of the isocyanates, and total RNA was isolated from draining lymph nodes (LN) 14 days post challenge. RPA analysis showed that all 4 isocyanates tested induced cytokines characteristic of a Th2 T-cell response (IL4, IL10 and IL13). These data support previous studies indicating that HMDI and IPDI are in fact respiratory sensitizers. (This abstract does not reflect EPA policy. This work was supported in part by The Dow Chemical Co. & DuPont Co.)

1184 AIRWAY RESPONSES AFTER SPECIFIC CHALLENGE OF RATS SENSITIZED VIA SKIN EXPOSURE TO TRIMELLITIC ANHYDRIDE (TMA).

X. D. Zhang, J. S. Fedan, D. M. Lewis and P. D. Siegel, ASB, NIOSH, Morgantown, WV.

TMA is a low-molecular-weight chemical that can induce production of specific IgE and occupational asthma in sensitized individuals. The respiratory tract is considered to be a major exposure route leading to immunological and airway sensitization, but the relationship between dermal exposure and subsequent airway responses to TMA is not known. Our previous study, using brown Norway (BN) rats, demonstrated that topical skin exposure to dry TMA powder induces dose-dependent production of specific IgE. The present study investigated the airway responses to inhale TMA of BN rats that have been sensitized dermally with TMA. Twenty mg of dry TMA powder was applied to the rats’ back (clipped with scissors) on days 0, 7, 14 and 21, followed by overnight with surgical tape. Rats were challenged by a 10 min, 40 mg/m3 TMA aerosol inhalation on day 35. Embryonic airway narrowing was recorded overnight in a whole body plethysmography system. Compared to non-sensitized rats, the sensitized BN rats displayed distinctive early (EAR) and late airway responses (LAR), as noted by an increase in Penn after TMA inhalation. The EAR occurred immediately following the challenge and lasted approximately 0.5 to 1 hour. This was followed by 1 hour of normal breathing. The LAR began from 3 to 4 hours after challenge and the increase in Penn lasted up to 19 hours following exposure. This work and our previous studies demonstrate that dermal exposure to TMA powder can lead to both immunological sensitization and obstructive airway responses upon aerosol challenge. This BN rat model, with both EAR and LAR, may be valuable for further study of pathophysiological mechanisms of organic acid anhydride-induced asthma.

1185 CHEMICAL AND VEHICLE RESPONSES TO TRIMELLITIC ANHYDRIDE (TMA) AFTER INTRATACHEAL (IT) CHALLENGE OF SENSITIZED MICE.


Exposure to some low molecular weight (LMW) chemicals has been known to produce respiratory hypersensitivity (RH), especially in occupational settings. Methods to predict the RH potential of new chemicals are needed by the regulatory community. While mouse models for RH to proteins are available, mouse models for LMW chemicals have not been developed. Our goal was to assess the effects of IT-exposure of TMA (free or conjugated) in selected vehicles. We evaluated lung responses to the known respiratory sensitiser TMA by dermally sensitizing and IT challenging mice. The contact sensitiser, 2,4-dinitrofluorobenzene (DNFB) was used as a negative control. Abdomens were exposed to 25% TMA for 2 consecutive days for 2 weeks. In week 3, TMA was applied to the ears. Two weeks later mice were IT-exposed. TMA in Hanks Balanced Salt Solution (TMA-HBSS), TMA in olive oil (TMA-O), mouse serum albumin-TMA conjugate in HBSS (MSA-TMA), Serum and bronchoalveolar lavage fluid (BAL) were collected before (D 0) and 1, 2, 3, and 7 days post IT (D1). TMA-sensitized mice challenged with TMA-HBSS had an increase in BAL total IgE at D1 and D2. Serum total IgE increased in TMA-HBSS and MSA-TMA IT groups as compared to controls. While DNFBB-HBSS challenge did not increase in serum total IgE, an increase in the MSA-DNFBB challenged group suggests that once conjugated to a protein, even non-respiratory sensitizers may stimulate IgE. Serum IgE increased in agent-oil and oil alone at D 7 in non-sensitized groups only, indicating that oil IT alone can evoke an independent serum total IgE increase. Our results suggest that none of these methods for delivering TMA to the lung were entirely satisfactory. It is clear that attention is needed in the area of chemical structure, stability, solubility, chemical-vehicle compatibility and compatibility with the biological system to develop appropriate models for the evaluation of LMWTH chemicals for RH potential. (This abstract does not reflect EPA policy.)

1186 CD11c/CD8α POSITIVE DENDRITIC CELLS FOR THE IDENTIFICATION OF CONTACT SENSITIZERS.

N. J. Gilmore, D. A. Baskette, B. C. Hulet, C. A. Ryan and G. F. Gebreker, SEAC, University of Louisville Laboratory, Sharnbrook, United Kingdom and Proctor & Gamble, Miami Valley Laboratories, Cincinnati, OH.

The use of human blood derived dendritic cell (DC) cultures has been examined as an in vitro alternative for the identification of contact sensitizers. Although often with variable outcome and not always with evidence of specificity. Thus, we aimed to derive a purified 'Langerhans cell (LC) like' DC population, a cell type more relevant for the identification of skin allergens. Pure populations of DC were isolated from human peripheral blood using BDCA-1 magnetic bead separation. The cells were cultured for 5 days in a combination of granulocyte macrophage colony stimulating factor, interleukin-4 and transforming growth factor beta to generate LC-like cells. The cells were generated approx. 95% CD11c/CD8α positive, expressed HLA-DR but not CD86 or CD83 as determined by flow cytometry, demonstrated endocytic ability (FITC-dextran uptake), were weak stimulators of the mixed lymphocyte reaction and thus could be regarded to possess an 'immature' phenotype. Cells obtained by this method (from either human peripheral blood or leucocytes) were exposed to subtoxic doses of the potent contact sensitizer DNCB (2,4-dinitrochlorobenzene). This resulted in elevated expression of HLA-DR (2.7 fold increase in mean fluorescence intensity (MFI)) and CD86 (15-20 fold increase in MFI) compared with control cells or those treated with subtoxic doses of the irritant sodium lauryl sulphate. Culture of blood derived CD11c+ dendritic cells thus may provide a population of Langerhans like cells for potential future in vitro approaches for the identification of skin sensitizers. Further investigation will determine whether this approach will provide a predictive model that stands up to future challenges of specificity and sensitivity.

1187 EVALUATION OF ANTIGEN SPECIFIC IgE RESPONSES IN C3H/HeJ MICE EXPOSED WEEKLY BY ORAL GAVAGE FOR SIX WEEKS WITH COW'S MILK PROTEIN AND CHOLECALCIFEROL.

R. S. Scott, C. S. VanPelt and G. S. Ladics, DuPont Co., Haswell Laboratory, Newark, DE.

The objective of this study was to evaluate the antigen specific IgE Dose-Response in mice intragastrically exposed to cow's milk protein (CMP) and cholecalciferol (CT) over a six-week period and to compare the responses to a cow's milk hypersensitivity model published by Li et al. (J Allergy Clin Immunol 103:206-214, 1999). Three-week-old female C3H/HeJ mice were sensitized by oral gavage to CMP plus CT, and boosted 5 additional times at weekly intervals. Serum was collected at weekly intervals and the level of casein specific IgE measured by ELISA. Test groups (n=7 to 8) included naive controls, 0.1 mg/g CMP + CT, 1 mg/g CMP + CT, 2 mg/g CMP + CT, 1 mg/g CMP without CT, and a CT only control. CT was administered at 0.3 μg/g. Casein specific IgE concentrations were determined by comparing individual or pooled sera samples against a standard curve. All analyses were performed in duplicate. As reported by Li et al., the maximal response occurred in the 1.0 mg/g CMP + CT group. However, differences in several measurements also occurred. We observed statistically significant differences in casein specific IgE levels between the 1.0 mg/g CMP + CT group and
the control groups by week 3 (Li et al. reported significant responses by week 6). Additionally, sera from 8 cases of specific responders were measured in all groups, including controls. The 1.0 mg/ml CMP + CT group had the highest fraction of responders and the highest pooled casein-specific IgG concentration. The number of responders and strength of response observed in the control group illustrated that some animals within each group were generating significant IgG titers specific for casein. Following these unexpected results, we learned that the standard animal feed used in our facility contained a proprietary amount of casein as a dietary supplement. Our results generally support these reported by Li et al. and illustrate the need to strictly control the possible sources of protein exposure when conducting animal hypersensitivity models.

1188 NEGATIVE CONTROLS FOR PROTEIN ALLERGENIC STUDIES.
NIHUEERL, USEPA, Research Triangle Park, NC, and College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

We have previously demonstrated immune and pulmonary responses typical of allergic responses in BALB/c mice exposed to fungal extracts of Metarhizium anisopliae crude antigen (MACA). MACA is composed of equal protein concentrations of the three component extracts from mycelium and conidia as well as indole-derived enzymes. Initial identification of the IgE-inducing protein(s) by SDS-PAGE analysis suggested that the conidial extract does not contain significant allergenic proteins. In order to further explore the possibility of a non-allergenic extract we exposed BALB/c mice to 4 aspirations (ASP) of 10 µg of MACA, Conidia extract (Comed), bovine serum albumin (BSA) (a weak or non-allergen control) in 50 µl Hank’s Balanced Salt Solution (HBSS), or HBSS alone over a 4-week period. Serum and bronchoalveolar lavage fluid (BALF) were harvested before (D0), and at 1 (D1) and 3 (D3) days following the final ASP exposure. MACA exposed mice had elevated levels of both BALF total protein (D1 and D3) and lactate dehydrogenase (LDH) (D1). However, neither Conidia nor BSA exposed mice had increased levels of these specific parameters, BALF differential cell counts demonstrated that Conidia exposed mice had substantial increases in neutrophils, lymphocytes, and eosinophils compared to HBSS and BSA exposed animals. However, these increases represent low cell counts when compared to MACA exposed mice. Additionally, there was little or no increase in the levels of serum total IgE for either the Conidia or BSA exposed mice. These data suggest that the M. anisopliae conidial extract contains one or more protein(s) that cause mild inflammatory responses but would be classified as a weak or non-allergen. It appears that the conidial extract might be a useful negative control when assessing fungal extracts of unknown allergenicity in the mouse model. (Supported by NCSU/EPA Cooperative Training Agreement CR826512010). (This abstract does not reflect EPA policy.)

1189 ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTE SUBSETS FROM COMMON MAMMOSAUS (CALLITHRIX JACCHUS/JACCHUS) OF UK OR SWITZERLAND/JAPAN ORIGIN.

The common mammosau (Callithrix jacchus jacchus) represents a recent addition to the primate species employed in preclinical safety studies in North America. The variability of lymphocyte subsets between mammals with different geographical origins is unknown. Source-related differences in lymphocyte subsets were investigated using immunophenotypic analysis of peripheral blood by flow cytometry to quantify differences between common mammosus of UK or Switzerland/Japan origin. Peripheral blood samples were collected from the conscious restrained mammals via the femoral artery or vein into trisodium citrate from 8 and 10 naive UK and Swiss/Japanese mammosus, respectively. Two-color assays were utilized for lymphocyte subset analysis to measure total T-cells, T-cell helper/suppressor cells, T helper cells, NK cells and B-cells. The procedure involved staining whole blood with monoclonal antibodies conjugated to two fluorochromes: CD20-fluorescein isothiocyanate (FITC), CD3-FITC, CD8-phycocerythrin (PE), CD2-PE, CD4-PE, and CD16-PE. The samples were analyzed on a Beckton Dickinson FACSCalibur, a flow cytometer equipped with a 488 nm argon laser. Preliminary results suggest there is no statistically significant difference in the percent of total T-cells, T helper/suppressor cells, T helper cells or B-cells between the UK and the Swiss/Japanese colonies. However, significant differences were detected between the NK cells (CD16+/CD3+) from the two colonies. Since immune modulators may potentiate the NK lymphocyte subset differences in the UK and Swiss/Japanese mammosus, consideration of animal source-related differences in biophysiologic measurements is critical to study design.

1190 DEVELOPMENT OF AN ALTERNATIVE TEST FOR PHOTOIRRITANTS: THE IN VITRO PHOTOTOXICITY ASSAY (IOPA).
D. R. Cerven, L. Y. Yukow, L. R. Kendall, T. L. Fox, T. L. Ripper and C. G. DeGeorge. MB Research Laboratories, Spiermont, PA.

The potential phototoxicity of pharmaceuticals, consumer products and cosmetics is of increasing concern to regulatory agencies and the public. Preclinical phototoxicity studies are typically performed using large numbers of experimental animals. The 3T3 fibroblast phototoxicity assay has been validated by the EU, but this and other in vitro tests lack the complexity, metabolic capability and predictivity of animal models. We have devised an In Vitro Phototoxicity Assay based on the well-characterized chorioallantoic membrane (CAM) vascular assay, which uses the fertilized chicken egg as a test system. Groups of eggs were administered increasing concentrations of known irritants, non-irritants, and phototoxins, 1-3/5 cm UVA irradiation. Eggs displaying vascular hemorrhage of the CAM, altered cell proliferation, or death of the embryo were scored as positive responses, and the percent positive eggs was plotted to determine the EC50 and EC90, from the respective log-Dose-Response curves. The Photo-Irritancy Factor (PIF = EC50/EC90) was calculated and a PIF > 5 indicated a positive phototoxic response. Using this assay, the phototoxicity of prototypical phototoxins 8-methoxypsoralen, promethazine, chlorpromazine, and 6-methyluracil were identified as phototoxic (RMOP; EC50 = 0.0003 mg/ml > PMZ; 0.0017 mg/ml > CPZ, 0.006 mg/ml > 6-MC 0.44 mg/ml), whereas SLS, dinitrochlorobenzene, benzalkonium chloride, ethanol and mineral oil were negative. In addition, the IOPA correctly identified the metabolically-activated prototypical photoirritants nadibumene and 5-aminovaline acid, which are phototoxic in vivo (due to hepatic metabolism) but not when assessed in vitro. In these cases, up to an 18 hour pre-treatment time prior to UVA irradiation was necessary to allow for distribution and conversion to the phototoxins. In conclusion, we have developed an alternative phototoxicity assay that allows rapid, inexpensive and sensitive screening of chemicals, while retaining many advantages of animal models.

1191 ASSESSMENT OF OCULAR IRRITATION RANGES OF MARKET-LEADING COSMETIC AND PERSONAL-CARE PRODUCTS USING AN IN VITRO TISSUE EQUIVALENT.

The cosmetics and personal-care industry has focused considerable effort on the search for replacements for traditional animal-based safety tests. in vitro models have been found to be particularly useful for the assessment of eye irritation potential. We have used the MatTek EpiOcular® OCL-25 tissue model (composed of human epidermal keratinocytes that differentiate and form a stratified squamous epithelium similar to corneal tissue) to establish ranges of in vitro ocular irritation scores for several categories of cosmetic and personal-care products. A significant advantage of the EpiOcular model is that it can be used to discriminate between the irritation potential of extremely mild products (most cosmetic and personal care products are formulated for inherent low irritation potential). Materials were topically applied to the EpiOcular tissue equivalents (167µL/cm2). Surfactant-based products were tested at a concentration of 10% to simulate "rinse-off" exposure; all other products were tested without dilution. Materials were tested using either the standard (4 hours) or extended (24 hours) exposure protocol (based on their expected irritation potential (ie. consideration of formula components, product type, etc.). Cellular viability was used as a marker for irritation potential and measured at various time points by a MTT metabolic colorimetric assay. MIT was quantitated spectrophotometrically at 570nm and an E100 (time to 50% loss of viability) calculated for each product. A range of in vitro ocular E100 scores was then determined for each product category. The results obtained from this study provide a reference database of in vitro ocular irritation scores for a cross-section of currently marketed cosmetic and personal-care products. Comparison of the E100 for new formulations with the range of E100 scores established for currently marketed products of similar type is a useful benchmark of anticipated consumer acceptability under conditions of actual use.

1192 THE APPLICATION OF BENCHMARKS FOR THE EVALUATION OF THE POTENTIAL OCULAR IRRITATION OF AEROSOL FRAGRANCES.

Aerosol fragrances are complex mixtures used in many consumer products. In aerosol air care formulations, organic solvents, such as ethanol, are added to the final formulation as solubilizers, stabilizers, fillers, and fragrance delivery mechanisms. The current product development/quality program was established to preclude routine in
**1193**

**EXPLORATORY IN VITRO EYE IRRITATION STUDY OF MARKETED ALKALINE LIQUID LAUNDRY DETERGENTS BY BCOP ASSAY AND PH/RESERVE ALKALINITY (RA) PARAMETERS.**

K. Cutr, T. Nussir, J. Merill, and J. Harrell. "The Dial Corporation, Scottsdale, AZ; Risk Assessment & Toxicology Services, Cincinnati, OH and 17630, Gaithersburg, MD.

An exploratory in vitro eye irritation study of 17 currently marketed alkaline liquid laundry (ALL) detergents was conducted to investigate the correlation between an in vitro biological endpoint and pH/RA ranges of ALL detergents. Currently marketed products can be considered "safe benchmarks," since they are produced by industry leaders and are assumed to have acceptable pH/Ra characteristics. Based on performance in previous in vitro eye irritation studies with surfactants and the potential to measure depth of injury, the bovine corneal opacity and permeability (BCOP) assay was selected as an in vitro endpoint to evaluate biological effects. Based on preliminary studies, a 25% (w/v) aqueous dilution of each detergent was applied to the corneas for a 20-minute exposure period. The degree of epithelial damage is reflected in the increase in fluorescein permeability value. Permeability values (OD490) for the 17 ALL detergents ranged from 0.28 to 2.193, reflecting a wide range in epithelial damage. The range of opacity scores was much lower (0.6 - 14.3) and showed little consistent change with the permeability values. Anionic/nonionic surfactant formulations often produce little in vivo opacity. The pH of each detergent was measured and ranged from 7.5 to 11.9. The RA (buffering capacity) was determined for a 1% (w/v) aqueous solution of each detergent stirred to a target pH of 9.5. Titration values ranged from 27.5 to 200 μmol of NaOH 0 to 120 μmol of HCl. Neither pH nor RA values correlated with the epithelial damage as measured by permeability changes. These data suggest that characteristics of the formulation other than pH or RA are responsible for the epithelial damage produced in the BCOP. This BCOP protocol, using a 25% (w/v) aqueous dilution with a 20-minute exposure, the permeability endpoint (with histological confirmation) and benchmark formulations, shows promise for evaluating ALL detergents.

**1194**

**USE OF HISTOLOGIC EXAMINATION IN BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY FOR ASSESSING THE OCULAR IRRITATION POTENTIAL OF FRAGRANCED FORMULATIONS.**


Fragrances are complex mixtures containing numerous chemical compounds and are referred to generically in the context of consumer product formulations. Aside from the inherently complex contribution of fragrance to a formulation's ocular irritation potential, it is also difficult to elucidate the role of other components in the mixture. This study was undertaken to evaluate the use of histological analysis to identify ocular damage not detectable by BCOP alone. Formulations were grouped according to base and fragrance type/concentration and comparisons were made between base-only and a series of fragranced formulations using the same base. Ocular irritation was measured using a standard BCOP assay with isolated corneas exposed to the material for 10 minutes, followed by opacity and permeability determination and histological analysis. Ethanol was used as a concurrent positive control. Within a series, BCOP scores varied significantly across fragrance type, ranging from 0.1 to 44.8, due mainly to changes in permeability. Histological analyses confirmed the relative degree of damage. For a given base, predicted irritation potential was dependent on the particular fragrance mixture. This study reinforces the need to determine the ocular irritation potential of formulations and not rely solely on component information. Understanding the nature and degree of ocular irritation potential to complex mixtures may help to improve the selection process for base and fragrance components of consumer products.

**1195**

**THE RABBIT ENucleATED EYE TEST (REET) AS A SCREEN TO REDUCE THE USE OF ANIMALS IN WORKER SAFETY STUDIES.**

F. J. Guerticke, C. W. Seaman, T. J. Sutton, C. J. Swagget, R. Guess and A. Whittingham. "Corporate Environment, Health and Safety, GlasscockSmithKline, King of Prussia, PA; Corporate Environment, Health and Safety, GlasscockSmithKline, Welwyn, United Kingdom and SafePharm Laboratories, Ltd., Derby, United Kingdom.

Identification of chemicals that cause eye irritation in the work place has traditionally used the Draize eye protocol. The REET, an in vitro alternative, shows promise for predicting severe eye effects. Test material is placed onto enucleated eyes (animals euthanized for other purposes) in chambers perfused with warm saline and washed off after 10 minutes. Corneal opacity, thickness, and fluorescein dye uptake are measured and corneal surface examined. Thirty materials (pharmaceuticals, intermediates, raw materials) were tested in both the Draize and REET. The Draize test identified 8/30 chemicals as severe with an additional 1 identified based on pH/visual effects. Overall, 13/30 chemicals were classified severe and labeled with EU phrase R41 - Risk of Serious Damage to Eyes. REET evaluation suggested that a cutoff score for any one of these parameters appeared predictive of the Draize response: corneal opacity/area ≥ 3; corneal swelling ≥ 25; fluorescein dye uptake/area ≥ 0.4 or observations of pitting, motling or sloughing. The REET-positive criteria were met by 17/30 materials and included 13 labeled R41 (100%) with 4 false positives (1 moderate, 3 mild). Thirteen chemicals did not meet the REET criteria: 9 were minimal/mild eye irritants and 4 were moderate. In conclusion, using the REET assay with pre-assigned cutoffs appeared to identify severe eye irritants and provided data for hazard labeling. When used as a pre-screen for the Draize assay, the REET contributes to reducing the number of animals used to test for eye irritants.

**1196**

**DEVELOPMENT OF AN IMMORTALIZED Z310 CHORDOR PLEXUS CELL LINE FOR DRUG TRANSPORT AND TOXICOLOGICAL STUDY OF BLOOD-CSF BARRIER.**


The blood-CSF barrier (BCB), located mainly in the epithelium of the choroid plexus, plays a wide range of roles in brain development, maturation, aging process, endocrine regulation, and pathogenesis of certain neurodegenerative diseases. To facilitate in vitro study of the BCB, we have used a gene transfection technique to immortalize murine choroidal epithelial cells. A viral plasmid (pSVneo) was inserted into the host genome of primary choroidal epithelial by calcium phosphate precipitation. The transfected epithelial cells, i.e. Z310 cells, that survived from cytotoxic selection expressed SV40 large-T antigen throughout the life span, suggesting a successful gene transfection. The cells displayed typical polygonal epithelial morphology by light microscopy. Immunocytochemical studies of transfected cells demonstrated the presence of transferrin receptor (TTR), a thyroid transport protein known to be exclusively produced by the choroidal epithelium. Western blot analyses further confirm the production and secretion of TTR by these cells. The mRNAs encoding transferrin receptor (TTR) were also identified by Northern blot analyses. The cells grew at a steady rate, currently in the 110th generation with a population doubling time of 20-22 h in the established culture. When Z310 cells were cultured onto a Transwell apparatus, the cells formed an epithelial monolayer similar to primary choroidal cells, possessing distinctive features such as an unique fluid level between inner and outer chambers and an electrical resistance approximately 150-200 ohm-cm². Moreover, the cells actively transported 3H]IT across this monolayer. These results indicate that immortalized Z310 cells possess the characteristics of choroidal epithelium and may have the potential for toxicological research. (Supported by NIH/NIHES ROI ES08146)
A proposed mechanism for the induction of peripheral neuropathies involves effects on mitochondrial dynamics, a process integral to neurite outgrowth (NG) and to the maintenance of axon and dendrite terminals. For modeling nerve growth and arbors, the sympathetic DRG isolated from fetal rats can be cultured to promote NG. In this investigation, we evaluated fetal-rat, dissociated and whole-embryo DRG culture systems using several toxicities with distinct mechanisms of action including glucagon polymerization/depolymerization, DNA alkylation and topoisomerase-III inhibition. In the dissociated DRG model, cultures were prepared in 96-well plates and evaluated via fluorescence-labeling and automated image analysis (ArrayScan). Whole-embryos were prepared in multi-chambered slides and evaluated by light-microscopy and analyzed via Quantitative image analysis. A viability assay was performed in each culture system to assess cytotoxicity. Both DRG models were employed to demonstrate concentration-dependent inhibition of NG in evaluation of: 1) compounds known to cause peripheral neuropathies in humans and 2) compounds not associated with neuropathic effects. In doing so, we showed that both test systems could be used to distinguish between specific and non-specific neuropathic agents by combining a viability assay with image analysis techniques. The effects of various concentrations of nerve growth factor (NGF) were evaluated in both culture systems to demonstrate potent neuroprotection. In the whole-DRG model, we showed that elevated concentrations of NGF (20-40 ng/mL) inhibited NG; as has been described previously. Interestingly, the dissociated-DRG culture system was relatively non-responsive to NGF concentrations between 2.9 and 900 ng/mL. Finally, we compared the relative merits of each culture system and the image analysis techniques carried out for both, and conclude that either system can be useful for evaluating compounds likely associated with peripheral neuropathy.

EARLY ENDPOINTS FOR EVALUATING CADMIUM-INDUCED IN VITRO NEPHROTOXICITY.


In vitro nephrotoxicity of cadmium chloride (CdCl2) on the proximal tubular epithelium, in vivo, increases the permeability of epithelial surface, while in vitro, it acts on active transient epithelial ion transport. The purpose of our research was to investigate the effects of CdCl2 on a porcine renal tubular cell line (LCL-PTK). In particular, we aimed to study the toxicity of CdCl2 on the brush border by measuring trans-epithelial resistance (TER) and paracellular permeability (PCP), and the molecular mechanisms underlying CdCl2 toxicity (by measuring metallothioneins [Mts], heat shock proteins [HSPs], reactive oxygen species [ROS]), and the induction of apoptosis. After exposure of the cells to different concentrations of CdCl2, TER decreased, while PCP increased in a concentration-dependent and time-dependent manner, indicating a structural alteration of the junctional complex. At the molecular level, we observed an increase in protective proteins, such as HSP70, from exposure to 10 μM and 25 μM CdCl2, respectively. Production of ROS at the mitochondrial level was also evident, indicating cellular oxidative stress. Treatment of the cells with higher concentrations of CdCl2, (100 μM) revealed a clear induction of apoptotic cell death. Our data indicate that CdCl2 nephrotoxicity can already be detected at rather low concentrations by measuring TER (at the barrier level) or Mts, HSPs, ROS production (at the cellular level), when cytotoxicity assays are unable to show any real damage. Therefore, these endpoints should prove to be very useful in studying xenobiotic-induced acute toxicity.

CULTURES OF EXFOLIATED HUMAN UROTHELIAL AND RENAL TUBULAR CELLS.

J. Müller1, A. Dörrenbach1, K. Goll1, P. Jendrusik1, H. Schulze1 and W. Föllmann1. Institute of Occupational Physiology at the University of Dortmund, Dortmund, Germany and Department of Urology, Stadtklinik Dortmund, Dortmund, Germany.

Since tissues of the urinary tract or the renal tubule from surgery or transplant donors have very limited availability, a promising source for these cells is the urinary sediment. Midstream urine samples from 24 adults were concentrated to sediments by repeated centrifugation (1000 x g, 5 min). To reduce urine concentration, sediments were washed twice with Ham's F12 culture medium. After resuspension in 0.5 ml culture medium, aliquots of 50-250 μl cell suspension were seeded onto collagen-coated 24-well plates. Cell culture was performed in a humidified incubator according to standard conditions. Vital cells started proliferation within 1 week after seeding and formed colonies of different morphology designated as type-1 or type-2 cell colonies. Most frequently, type-1 cell colonies occurred which showed irregular colony contours and randomly arranged spindle-like cells. Subcultivation was possible up to 6 passages. Immunohistochemical staining for the epithelial marker cytokeratin-7 was positive. Staining for carbonic anhydrase was negative. Type-2 cell colonies which could be subdivided into two subgroups by morphological characteristics only occurred occasionally. These colonies showed smooth-edged contours. A subcultivation was not possible. Cobblestone-like type-2 cells were able to form domes. These cells were positively stained for carbonic anhydrase, indicating a tubular origin. Primary cell cultures of exfoliated cells from urinary sediments can be used for in vitro studies in toxicological and clinical research.

SUBACUTE CYTOTOXICITY TESTING WITH CULTURED HUMAN LUNG CELLS.

K. A. Barrie, A. Yang and D. L. Cardona, College of Pharmacy, Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY.

This study was designed to evaluate the potential of an in vitro cell culture method for its ability to determine subacute cytotoxicity and to compare the cytotoxic concentrations with rodent L50s and clinical human toxicity data. Human fetal lung fibroblasts (HFL1) were incubated in the absence or presence of increasing concentrations of test chemicals for 72 hours, and cell proliferation was used as a marker for toxicity. Inhibitory concentrations were extrapolated from concentration-effect curves after linear regression analysis. Comparison of the cytotoxicity data from testing 50 chemicals, with available human lethal concentrations for the same chemicals, revealed that the 72-hour experimental IC50s are as accurate predictors of human toxicity as equivalent toxic blood concentrations derived from rodent L50s. In addition, subacute 72-hour exposure of HFL1 cells more accurately predicts cytotoxicity than a 24-hour microsomal assay previously conducted in our lab, although the experimental IC50 values were not statistically different in the two assays (table below of first 15 chemicals). It is anticipated that this procedure, together with a related battery of tests, may supplement or replace currently used animal protocols to screen chemicals for human risk assessment.

**Table**

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<th>Number</th>
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INHIBITION OF FATTY ACID ETHYL ESTER SYNTHASE ACTIVITY BY TRI-O-TOLYPHOSPHATE METABOLITES IN HEPG2 CELLS.

K. A. Meradle, B. S. Kaphalia and G. A. Ansari. Pathology, University of Texas Medical Branch, Galveston, TX.

Fatty acid ethyl ester synthase (FAEES) is a family of enzymes that catalyzes the conjugation of alcohols and amines with fatty acids. Resultant conjugates are shown to be highly lipophilic, which can accumulate in target organs and cause toxicity. Previous studies from this laboratory have shown that FAEES purified from rat liver is structurally and functionally different from that of pancreas, and only hepatic FAEES activity is inhibited by tri-o-tolypophosphate (TOTP, a serine esterase inhibitor). Metabolism of TOTP is prerequisite for such inhibition. Therefore, two known metabolites of TOTP, 2-O-(4-ethyl)-4-hydroxy(benzophenone-4-one (CBDPD) and hydrolyzed TOTP [4-O-(de-tolyloxy)-4-hydroxy]tolphosphate were synthesized and their inhibitory effect on FAEES activity was investigated in HepG2 cells (human hepatoma cell line). HepG2 cells in culture were incubated with an equimolar concentration of 2.5 μM TOTP CDPD or hydrolyzed-TOTP.
in ethanol at 37°C (a concentration causing 85% inhibition by CBP in preliminary experiments). Cells treated with equivalent amount of ethanol served as control. Cells were harvested after 1 hour exposure, washed, scraped and homogenized. FAEEs and p-nitrophenylacetate (PNA) hydrolyzing (esterase) activities were measured. Inhibition of FAEEs as well as esterase activities was found to be greatest in the cells treated with CBP and hydroxylated-TOTP (-85% and 59%, respectively), followed by TOTP. The time course study performed by TOTP showed that inhibition by CBP occurs in 15 minutes (earliest time point investigated). We conclude that the active metabolites of TOTP (CBP and hydroxylated-TOTP) cause the inhibition of FAEEs and esterase activities in HepG2 cells.

1202 MECHANISMS OF CERAMIDE-INDUCED APOPTOSIS IN A RAT HEPATOMA (H4IIE) CELL LINE.


Ceramides are a group of sphingolipids that act as second messengers in the control of many biochemical functions including apoptosis. Tumor necrosis factor alpha (TNF) can increase the hydrolysis of sphingomyelin to form ceramide. Both ceramide and TNF have been shown to cause apoptosis via activation of caspases. Ceramide has also been shown to induce apoptosis via pathways independent of caspase activation. Thus, the precise signalling pathways activated by ceramide depend on the cell type. If TNF-activated apoptosis is dependent on the formation of ceramide, then ceramide and TNF should produce similar effects in vitro. In the present study the biochemical pathways involved with ceramide-induced apoptosis were compared to those of TNF in a rat hepatoma (H4IIE) cell line. Cells were seeded at 10,000/200 µl in 24-well plates and equilibrated for 48 hr, and then exposed to C2-Ceramide (0, 0.01, 0.1, 5, 10, 50, 100, and 300 µM) or TNF (0.0, 0.5, 2.5, 5, 25, 75, and 150 ng/ml) with specific activity = 1.0 x 10^7 U/mg for 24 hr. Following the exposure period, changes in cell health were determined by monitoring mitochondrial membrane leakage, and cell proliferation. Oxidative stress was assessed by measuring changes in reduced glutathione (GSH) and membrane lipid peroxidation. Apoptosis was evaluated by caspase (3 and 9) activity and DNA fragmentation. TNF was cytotoxic above 5 ng/ml; however, there were no significant changes in total GSH prior to cell death or in membrane lipid peroxidation as determined by 8-isoprostane. Exposure to TNF caused a marked increase in caspase 3 and 9 activities as well as DNA fragmentation, but did not increase lipid peroxidation. In contrast, ceramide had no effect on caspase 3 or 9, but did increase lipid peroxidation (10-300 µM ceramide). C2-ceramide also increased DNA fragmentation at the highest exposure (300 µM) and reduced mitochondrial function. These data suggest that in H4IIE cells apoptosis induced by TNF may occur through signalling pathways that are different from those induced by C2-Ceramide.

1203 HALOGENATED HYDROCARBON TOXICITY IN RAT PRIMARY HEPATOCYTES: INDUCTION OF OXIDATIVE STRESS AND DEVELOPMENT OF A NOVEL TOXICITY RANKING METHOD.

K. T. Geiss and J. M. Frazier, Operational Toxicology Branch, AFRL/HEST, WPAFB, OH.

Volatile halogenated aliphatic compounds are among those chemicals that can cause oxidative stress in vitro and in vivo. Here, we investigated the effects of 20 brominated and/or chlorinated one- or two-carbon molecules, including carbon tetrachloride, trichloroethylene and 1, 1, 1-trichloroethane. Following the use of a unique in vitro methodology to expose primary rat hepatocyte cells to these volatile chemicals, biological responses were assessed from separate exposures of rat primary hepatocytes to these halogenated aliphatics. Indicators of lipid peroxidation, reactive oxygen species, catalase enzyme activity, cellular thiol status, and cytotoxicity were measured. For this large group of chemicals, classification by multiple endpoints for oxidative stress in primary rat hepatocytes has not been shown previously. The toxicity values for these chemicals, such as the EC50s, ranged from 0.10 to 127.00 mM. We derived a novel composite toxicity index for purposes of ranking the potential toxicities of the chemicals. This scoring index had a potential range from 6 to 130, for ranking purposes. In this study, the composite scores ranged from 11 to 115. We also derived QSAR models to describe the relationships for these chemicals between their physico-chemical properties and their potential to cause oxidative stress in vitro. Our results show the ability to rank a group of chemicals, based on their in vitro toxicity values for multiple endpoints and the effectiveness of QSAR models in predicting relative toxicity ranking of chemicals. The impact of this approach provides a means of disentangling the relative toxic potencies among a group of chemicals, which could be used in materials or drug development or identification of relative risk.

1204 RANKING GLITAZONE COMPOUNDS FOR HEPATOTOXIC POTENTIAL BY MONITORING STRESS-SPECIFIC ALTERNATIVE mRNA ISOMORPHS: EVIDENCE AGAINST A GLITAZONE CLASS TOXICITY?


A low incidence of severe unanticipated hepatotoxicity resulted in troglitazone (TRO) withdrawal. The ability of gene expression profiling in the Safe-Hit DNA macro-array, representing a library of 682 stress-specific p53-induced mRNA isoforms, to correctly rank for toxic potential three commercially available glitazone compounds was evaluated. Primary cultures of human hepatocytes from eight donors and the hepatocarcinoma cell line HepG2 were incubated for 16 hr with TRO, pioglitazone (PIO) or rosiglitazone (ROS) at concentrations ranging up to approximately 20% cytotoxicity (MTT). The mRNA was isolated and used to probe the Safe-Hit macro-array. Up- or down-regulation of stress-specific mRNA isoforms by 2.5-fold relative to vehicle control cells was considered to represent gene deregulation, and a toxicity index corresponding to the total number of deregulated genes for that particular condition was calculated. TRO had the highest toxicity index in all cases and the difference was greater in the primary human hepatocytes than in HepG2. Toxicity indexes at the highest concentrations for TRO, PIO and ROS were 45, 11, and 7, respectively, in primary cultures of human hepatocytes and 33, 28 and 26, respectively, in HepG2 cells. The high toxicity index induced by TRO and the lower ones induced by PIO and ROS in human hepatocytes correlate with what is known about the clinical safety of these drugs and indicate that hepatotoxicity might not be a simple glitazone class-specific event. That the repertoire of deregulated genes differed between TRO on one side and PIO or ROS on the other further supports this idea. These results suggest that the monitoring of appropriately selected alternative mRNA isoforms in those in the Safe-Hit library, may be a method of choice for in vitro detection of compounds that will induce rare severe liver toxicity, a phenomenon that historically has been difficult to predict.

1205 ANTIDIABETIC DRUGS IN THE THIAZOLIDINEDIONE CLASS REDUCE GLUTATHIONE LEVELS AND INCREASE LIPID PEROXIDATION IN AN IN VITRO MODEL.


Thiazolidinedione (TZD), troglitazone (TRO), pioglitazone (PIO), and rosiglitazone (ROS) act in the TZD class of oral hypoglycemic drugs used in the treatment of diabetes. TRO has been shown to produce idiosyncratic liver toxicity. The mechanisms underlying TRO hepatic toxicity remain unknown. Metabolism of TZD drugs involves ring opening and subsequent conjugation with GSH. TRO has been shown to reduce GSH and induce oxidative stress. Patients with type II diabetes have a significant reduction in tissue GSH. Moreover, the ability of a tissue to protect itself from oxidative stress is dependent on many factors including diet, age, and exposure to other medications. Some diabetic individuals may be unable to adapt to compounds that produce oxidative stress by depleting GSH. The purpose of the present study was to evaluate the relative cytotoxic and oxidative stress potential of TRO, PIO, TRO, and ROS in an in vitro cell system. Rat hepatoma (H4IIE) cells were seeded into 96-well plates (10,000/200 µL). Following a 48 hr equilibration period the cells were treated with compounds at concentrations of 0, 0.1, 5, 10, 50, 100, and 300 µM for 24 hr. Cytotoxicity was evaluated by measuring membrane leakage, mitochondrial function, and cell number. Oxidative stress was assessed by measuring GSH and 8-isoprostane (8R). Markers of general toxicity were essentially unchanged at exposures below 100 µM. TRO and PIO reduced GSH and produced a concomitant increase in membrane lipid peroxidation. These changes were observed at low concentrations prior to changes in general cell health. TRO also reduced GSH levels, but only produced a modest increase in 8R at the highest exposure. ROS produced a 8R increase, but had no detectable effect on 8R. Thus, in terms of oxidative stress; TRO > PIO > TRO > ROS. These data suggest that the antioxidant status of individuals with diabetes may be a determining factor in how they respond to the TZD class of drugs.

1206 INCORPORATING LCMS IN DETERMINING CYTOCHROME P450 FORM-SPECIFIC ACTIVITY.


The goal of drug discovery organizations is to increase the probability of compound success in clinical trials. Currently, the three main reasons for clinical failure are lack of efficacy, unexpected toxicity and poor drug metabolism/pharmacokinetics. New
methods and technologies are constantly being incorporated into drug discovery to screen new chemical entities (NCEs) with regards to these three areas, especially in the area of metabolism. Metabolism screening techniques rely upon various in vitro test systems, such as microsomes, S9, hepatocytes, etc. In order to use these systems, the native activity of the drug metabolizing enzymes, primarily the cytochrome P450 (CYP450), must be determined. CYP450 activity is also used to determine the inhibition potential of NCEs. Inhibition of the NCE metabolizing enzymes could result in elevated blood levels and greater likelihood of toxicity. In order to increase screening throughput, a faster method to evaluate CYP450 activity was developed. Previous methods required incubating a CYP450 marker substrate with the test system and determining metabolic production via HPLC/GC detection. A new analytical method was developed using LC/MS/MS to evaluate the production of metabolites for six CYP450 forms (1A2, 2C, 2D, 7C9, 126, 3A4/5 and 4A11) following either multiple incubations or a single CYP450 "cocktail" incubation. The inhibition potential of SK-C35840 and VX-745 (0.5 to 25 µM) was determined using individual CYP450-specific substrate incubations as well as "cocktail" incubations of CYP450 marker substrates in pooled human liver microsomes. Regardless of incubation method, SK-C35840 inhibited the activity of CYP3A4/5 and 2D6, and VX-745 inhibited the activity of CYP2C19 but not CYP2D6. Individual incubations with either SK-C35840 or VX-745 decreased CYP3A4/5 activity but "cocktail" incubations with either compound had no inhibition towards CYP3A4/5.

1207
A SENSITIVE METHOD TO DETECT METABOLIC TOXICITY IN A HOMOGENEOUS CELLULAR ASSAY


A major concern in the pharmaceutical industry is the potential for metabolic toxicity of developmental compounds. However, existing assays are extremely cumbersome and time-consuming. Here, we present a cell-based, high throughput-screening assay that detects and differentiates between direct and indirect acting toxins. A β-lactobacillus cell line, MCL-5, was created expressing 5 human P450's known to be important in metabolic toxicity. CYP1A1, 1A2, 3A4, 2A6 and 2E1 and epoxide hydrolase. Direct and indirect toxicity was measured with the MCL-5 cells in comparison to its control cell line ch2 cells, using the BD Oxygen Biosensor System (OBS) as the method of detecting cell viability. Experiments were annotated and IC50's determined using the Multi-well Plate Manager (MPM) - ADMET software program. Data and the associated experiments were archived in MPM's internal database for easy retrieval. The MCL-5 cells were shown to be significantly more sensitive to compounds that required metabolic activation for toxicity such as NNN, aflatoxins and benzopyrene (Table). The detection of toxicity for compounds such as acetonitrile required the inhibition of glutathione by 50µM buthionine sulfoximine (startet compounds). Moreover, the metabolic toxicity of cytosphosphamide could be specifically inhibited by competitive inhibition of CYP2B6 by a non-toxic dose of coenzyme A. The OBS in combination with the MCL-5 and ch2 cell lines creates a simple, homogeneous, streamlined method for screening compounds for metabolic toxicity.

<table>
<thead>
<tr>
<th>Test Substrate</th>
<th>ch2 cells</th>
<th>MCL-5 cells</th>
<th>Major P450's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzopyrene</td>
<td>1.6µM</td>
<td>0.1µM</td>
<td>1A3, 3A4, 1B1</td>
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<td>0.007µM</td>
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<td>2.61µM, 2D6</td>
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<td>Acetaminophen*</td>
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<td>1.200µM</td>
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<tr>
<td>PhIP</td>
<td>111.2µM</td>
<td>21.28µM</td>
<td>1A1</td>
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<tr>
<td>Methotrexate</td>
<td>0.021µM</td>
<td>0.023µM</td>
<td>Direct-Acting</td>
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</tbody>
</table>

1208
INDUCTION OF CYTOCHROME P450 (CYP) FORMS IN CULTURED HUMAN LIVER SLICES.


Cytchrome P450 (CYP) inducibility is a major issue in drug development, as it reflects a potential risk of drug interaction. One promising in vivo model to predict CYP inducibility is cultured human liver slices. The region-specificity of CYP dis-
1211 MORPHOLOGICAL AND BIOCHEMICAL INTEGRITY OF HUMAN LIVER SLICES: EFFECTS OF OXYGEN TENSION.


We tested the effects of low (20% O2) and high (70% O2) oxygen tension on the morphological and biochemical integrity of human liver slices incubated for up to 72 h in a dual rotating culture system. High oxygen tension was more effective than low oxygen tension for preserving morphological integrity in long-term culture (48-72 h). After 72 h of culture with 70% O2, the lobular pattern was well preserved, and the survival of hepatocytes (approximately 80%) and other cell types was good. Immunohistochimical studies showed good preservation of the liver-specific expression of CYP 2E1 and 3A4 isoforms for up to 72 h of incubation in 70% O2. As compared to 20% O2, the oxidized glutathione content and reactive oxygen species production were slightly increased in 70% O2, suggesting that minimal oxidative stress occurred with the high oxygen tension. In conclusion, despite slight oxidative stress associated with high oxygen gas in the incubator, 70% O2 appeared to be optimal for preserving the morphological and biochemical integrity of human liver slices cultured in a dynamic organ culture system for up to 72 h. (Supported by EU Biotechnology Program, B104-CT97-2145).

1212 THE CYTOTOXICITY AND METABOLIC CHANGES INDUCED BY LEWISITE IN IN VITRO EXPOSED HUMAN LEUKOCYTES.

H. L. Meier, BiochemPharm, USA/RMC, Aberdeen Proving Ground, MD. Sponsor: S. Bankin.

To study the mechanism of vesicant for the development of countermeasures, the cytotoxic and metabolic effects of the potent chemical warfare vesicant, lewisite (arsole, dichloro (2-chlorovinyl) L) were investigated in a human leucocyte model. Human leucocyte preparations were isolated by Percoll centrifugation and suspended at a final concentration of 10^6 cells/ml in Tyrode’s buffer with either buffer or L for various times. To determine the cytotoxicity of L at concentrations between 2 x 10^-4 to 8 x 10^-4 M, the cells were incubated for 1, 2, 4, 8, 12, 16, 18, and 22 hrs post-exposure before propidium iodide was added. The percent of viable cells was measured by using flow cytometry. The concentration-dependent cytotoxicity of L was detected as early as 1 hr post-exposure in the range of 1 x 10^-4 to 6 x 10^-4 M. At the longer time intervals, cytotoxicity could be detected at lower concentrations of L. In the investigation of the metabolic changes of L, cells were exposed to various concentrations ranging from 6 x 10^-4 to 6 x 10^-5 M. After exposure, the cells were harvested by centrifugation and their ATP levels were measured by the LKB-Wallac 1243-107 ATP Assay Kit in the LKB-Wallac 1250 Lumimeter. Only lewisite exposed to 6 x 10^-4 M L demonstrated an initial decrease in ATP within 15 minutes after exposure. In a 24 hrs post-exposure, there was a concentration-dependent decrease in ATP. By 2 hrs post-exposure, the concentration-dependent decrease appears to be almost linear. The level of ATP then appears to plateau at all the concentrations of L studied from 4 to 24 hrs post-exposure. It appears that any assay designed to determine whether an antivenom antibody directed against L is efficacious should be given during the first hr of post-exposure to block the effects on both viability and metabolism.

1213 DEVELOPMENT OF IN VITRO ANTIGENICITY STUDY EXPOSING TO TEST COMPOUNDS IN IN VITRO PRIMING CULTURE BY USING DENDRITIC CELLS (DC) INDUCED FROM BONE MARROW CELLS (BMC) IN MICE.


In early stages of drug discovery, evaluations of toxicities are very important to reduce the attrition rate of the compounds in later stages of drug discovery and development. But, high throughput toxicity evaluation systems are not well established. In this paper, we investigated an in vitro antigenicity testing method by using mouse DC induced in in vitro culture system from BMC. BMC isolated from mice and cultured with various cytokines (granulocyte macrophage-colony stimulating factor (GM-CSF) + interleukin-4, GM-CSF + tumor necrosis factor alpha, or GM-CSF + interleukin-6 + IL-13 ligand) for 6 days. The cell numbers were increased during the culture period, and cells having characteristics of DC (dendritic morphology, expression of DC markers; CD 40, CD80 and CD86) were induced after 4-5 days of the culture. To confirm an antigen-presenting activity of the induced DC, the DC were exposed to positive reference compounds (antigens) in the culture system (a priming culture) for 1 day, and intraperitoneally injected into untreated mice for 2-times in a 2 week interval. At 1 week after the last injection of primed DC, sera were isolated for the measurement of positive reference compound-specific antibodies, and splenocytes were isolated for an in vitro lymphocyte proliferation test (response to the antigen). High antibody titers were observed in bovine serum albumin (BSA), and positive antibodies were observed in trimethobenzene sulfonic acid, sodium selenite (DNBS) and trimethylamine N-oxide. Splenocyte proliferations were observed for BSA, DNBS and penicillin G. These results show that functional DC which have an antigen-presenting activity were induced by the culture of mouse BMC with the cytokines. These DC would be a candidate cells for antigen-presenting cells in in vitro antigenicity test.

1214 RESPONSIVENESS OF MONKEY PLATELET AGGREGATION AGAINST THREE DIFFERENT AGGREGATION AGENTS.

K. Okazaki, Y. Morii, S. Neechev, D. LaFraamboisie, R. Coken, B. Congdon, K. Fukuzaki and R. Nagata. SNBL USA, Ltd., Everett, WA.

This experiment was designed to compare non-human primate platelet aggregation using three aggregation agents. These data were also compared to human platelet aggregation with the same agents. Twelve male and five female cynomolgus monkeys and seven male human volunteers supplied platelet specimens. Maximum platelet aggregation rates (MPAR) in monkey platelets, after the addition of Adenosine Diphosphate (ADP), Collagen (1.5, 10, and 20 μM), and Epinephrine (EPI; 300 μM), were evaluated by gender and compared to human data. Lipoprotein levels of monkey plasma were analyzed by electrophoresis and compared to human electrophoresis data. Both monkey and human MPAR increased dramatically after the addition of COL, and 10 or 20 μM ADP. Monkey MPAR was slightly increased after a 5μM ADP addition. Monkey MPAR showed a minimal response to an EPI addition in comparison to the human MPAR response. There was also no gender difference in monkey platelet aggregation induced with 20μM ADR, COL or EPI. It was previously reported that the MPAR response, after EPI treatment, was significantly less in cynomolgus monkey platelets as compared to human platelets (F.Med. Primatol.15, 1980). On the other hand, the influence of lipoproteins on platelet aggregation (Ateriosclerosis 3, 1983; Herrois 8, 1979; Prostaglandins Leukotrienes and Essential Fatty Acids 51, 1994) was also reported in humans. In this experiment, low-density lipoprotein (LDL) levels in monkeys were about two-fold lower than in humans. Different LDL levels between both species were considered as the possible cause of low sensitivity in monkey platelets against EPI. In conclusion, monkey platelets showed similar or different responsiveness against three aggregation agents when compared to humans.

1215 HEMOLYTIC POTENTIAL OF VEHICLES USED FOR RAPID IN VIVO SCREENING OF SMALL MOLECULES.


Vehicles that are administered parenterally in vivo may be screened in vivo to determine the degree of hemolysis in erythrocytes and to evaluate the effect, if any, of varying vehicle concentrations on hemolysis. This study assessed the hemolytic properties of various vehicles used for rapid in vivo screening of small molecules when exposed to rat blood in vivo. All the test articles (Tween 80, ethanol, hydroxypropyl β-cyclodextrin, polyethylene glycol 400, propylene glycol, polysorbat 188, dimethyl sulfoxide, dimethyl acetamide and multiple component vehicles) were diluted to the desired concentrations in phosphate buffered saline solution. Mixtures containing equal volume of blood and each vehicle dilutions were incubated under static conditions for 30 minutes at 37 ± 1°C. The tubes were centrifuged, and the supernatant harvested were observed visually for the appearance of red color and the optical densities were read at wavelength of 545 nm. A hemolytic index for each vehicle formulation was determined by comparing the optical density determined spectrophotometrically in the test article samples to the optical density under conditions of 0% and 100% hemolysis. There was a positive correlation between plasma color and optical density in all samples. Hemolysis was not seen at the following concentrations in these vehicles: Tween 80 ≤ 0.25%, Ethanol ≤ 5%, Propylene glycol ≤ 2.5%, Polyethylene glycol ≤ 5%, Polysorbat 188 ≤ 0.5%, Dimethyl sulfoxide ≤ 9% and Dimethyl acetamide ≤ 7.5%. All the multiple component vehicles (25% Ethanol-5% Cremophor EL, 10% DMAC-20% Ethanol, 10% DMAC-40% PG and 20% PG-20% PEG 400) induced hemolysis in rat blood. In vivo animal studies and in vitro cytotoxicity studies to understand the toxicity profiles of these commonly used vehicles are in progress.
1216 SELECTION OF REFERENCE CHEMICALS TO VALIDATE IN VITRO CYTOTOXICITY ASSAYS FOR ESTIMATING IN VIVO STARTING DOES FOR ACUTE ORAL TOXICITY.


The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICETAM convened an international workshop in October 2000 to evaluate the validation status of in vitro methods for predicting acute systemic toxicity (see http://iccam.nih.gov). As an interim step to developing and evaluating using in vitro cytotoxicity methods to accurately predict relevant oral LD50 for regulatory purposes, workshop participants recommended the use of in vitro methods to predict the in vivo LD50 starting doses in situations where other credible information was unavailable. It was conjectured that such an approach would further reduce the number of animals required for acute toxicity testing. In a joint effort with the USEPA, and in collaboration with the European Centre for the Validation of Alternative Methods (ECVAM), NICETAM is organizing a multi-laboratory study to validate the utility of two in vitro cytotoxicity tests for identifying the starting doses for in vivo acute toxicity tests. A major aspect of the study was the selection of an appropriate number of test chemicals that represent a wide range of toxicity, the types of chemicals regulated by the various US regulatory agencies, and those with human toxicity data and/or human exposure potential. Based on these criteria, a preliminary list of approximately 100 chemicals was compiled by mining several publicly available data sets. A priority list of 60 chemicals representing the 6 acute toxicity hazard classifications in the Globally Harmonized Scheme was created based on the comparability of the test systems, availability and quality of existing oral LD50 data, human exposure data, commercial availability, ease of shipping, and availability of information on mechanism of action. Supported by NIEHS contract N01-ES-85424 with ILS, Inc.

1217 PRELIMINARY EVALUATION OF A CYTOTOXICITY MODEL FOR PREDICTING ACUTE ORAL TOXICITY TESTING STARTING DOES.

G. C. Mun1, R. R. Rupple2 and R. D. Curren1, 1Institute for in vitro Sciences, Inc., Gaithersburg, MD and 2Borealis Corporation, Gaithersburg, MD.

For almost fifty years, in vitro cytotoxicity methods have been studied as predictors of acute lethality in vivo. Recently, Halle has compiled a Registry of Cytotoxicity (RO) which compares the LD50 (in mmol/kg; rat or mouse) of 347 chemicals with an average in vitro LC50 (in mmol/L; various cell types). A graph of the RO shows a reasonably good correlation between lethality in vivo and cytotoxicity in vitro for 73% of the points lie within a +/- log 5 interval around the regression line. This led Spielemann et al. to propose that, at a minimum, the results of in vitro basal cytotoxicity tests can be used to estimate the starting dose for in vivo LD50 tests. It has also been suggested that new cytotoxicity methods be first qualified for use in this scheme by testing them with RO chemicals having a range of toxicities and which fit closely to the RO regression line. To qualify, the new results should have a similar slope and lie within +/- log 5 of the RO regression line. We investigated whether two commonly used cytotoxicity tests (neutral red uptake in BALB/c 3T3 [3T3] and normal human keratinocytes [NHK]) would qualify under these conditions. Eleven chemicals from the RO whose in vivo LD50s range from -0.1 mmol/kg to >100 mmol/kg were chosen for evaluation. All chemicals were tested under code using a well-defined standard protocol. Cytotoxicity in the treated cells relative to that of control cells was determined after 24 hr (3T3) or 48 hr (NHK) exposure. When the resulting data were overlaid on the original RO graph, all new data points lay within a +/- log 5 interval around the RO regression line. The slopes of the regression lines for the new tests were 0.506 (3T3) and 0.498 (NHK) versus 0.625 for the RO. R2 values were 0.985 (3T3) and 0.936 (NHK). We conclude that the proposed qualification criteria are reasonable and that both of these candidate tests have met those criteria.

1220 TOXICITY TESTS USED ONLY AS SCREENS STILL NEED CAREFUL VALIDATION.

R. D. Curren1, J. W. Harbell2 and L. H. Brunet3, 1Institute for in vitro Sciences, Inc., Gaithersburg, MD and 2The Gillette Company, Needham, MA.

New toxicity tests, especially in vitro tests, are often used as screens; i.e., they are used to preliminarily divide a test set of chemicals into positive or negative subsets. One of two strategies can then be applied. Either 1) the negatives can be accepted as correct and the presumptive positives evaluated in a second tier test, or 2) the positives can be accepted and the negatives evaluated in a second tier test. There has been a tendency to assume that validation does not have to be as stringent for a screen as it does for a stand-alone test because the screen is only a preliminary assessment. However, both approaches 1) and 2) above use screens to make some firm decisions; the first to classify a chemical as non-toxic and the second to classify the chemical as toxic. It is often thought that sensitivity (fraction of known positives correctly identified) is the most important criterion and that specificity (fraction of known negatives correctly identified) has much less significance. It follows that sensitivity alone could be characterized by using predominantly positive chemicals in a validation study. We present data to show that this reasoning can lead to major errors. In approach 1), choosing a tier 1 test with specificity specified will result in a high number of false positives passing through the more expensive or animal intensive tier two test, possibly negating the advantage of having two tiers. This is especially relevant when several mechanistically specific tests are combined into a tier 1 battery. In approach 2), a low specificity tier 1 test means that many useful chemicals may be discarded without necessity due to the prevalence of positives in the subset of negative chemicals identified by the screen. Finally we show that using predominantly positive test materials to validate a screen can result in a serious overestimation of the test's sensitivity. For any test to be useful as a screening test or as a stand-alone test, it must be both sensitive and specific for the endpoint of interest.

1219 THE SENSITIVITY AND SPECIFICITY OF A TOXICITY TEST ARE DEPENDENT ON THE DISTRIBUTION OF TOXIC CHEMICALS BEING EVALUATED.

L. H. Brunet1, G. J. Carr1, J. W. Harbell2 and R. D. Curren3, 1Gillette Medical Evaluation Laboratory, The Gillette Company, Needham, MA, 2Miami Valley Laboratories, The Proctor & Gamble Company, Cincinnati, OH and 3Institute for in vitro Sciences, Gaithersburg, MD.

Often, the only measures of toxicity test performance provided after a validation study are the statistics sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and concordance. Sensitivity and specificity, which have been assumed to be independent of the distribution of test performance since NPV and PPV are commonly known to vary with changes in prevalence. The purpose of these studies was to test whether or not sensitivity and specificity are true constants. Monte Carlo simulations were used to generate four sets of data that fit a simple prediction model. The distribution of toxic chemicals was varied between four sets of data evaluated in the study. The distribution of toxicity in two of the data sets skewed such that either strongly or weakly toxic chemicals predominated. The toxicity of chemicals in the third data set was predominately strongly and weakly toxic with few chemicals of intermediate toxicity. The fourth set contained chemicals predominately of intermediate toxicity with relatively few strongly or weakly toxic chemicals. Cut-offs were set at different levels across the range of response and the performance statistics were calculated at each cutoff. The results showed that sensitivity and specificity, measured at identical cut-offs, were not constant across the data sets. We conclude that sensitivity and specificity should not be considered constant indicators of toxicity test performance and that more emphasis should be placed on the assessment of a toxicity test's positive and negative predictive value.

1220 RESULTS OF AN INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS (ICCVAM) EXPERT PANEL EVALUATION OF THE VALIDATION STATUS OF THE FROG EMBRYO TERATOGENICITY ASSAY-XENOPUS (FETAX) FOR IDENTIFYING DEVELOPMENTAL TOXICANTS.

R. R. Tice1, A. Aueter2, G. Dagon3, E. Fauman4 and W. S. Stokes4, 1NICETAM, ILS, Inc., Research Triangle Park, NC, 2OCTP, USEPA, Washington, DC, 3Procter & Gamble, Cincinnati, OH, 4Univ. of Washington, Seattle, WA and 4NICETAM, NIEHS, Research Triangle Park, NC.

One of the most important aspects of a toxicity assessment is the determination of the potential of a chemical to affect fetal development. Traditional in vivo rodent methods for evaluating developmental toxicity potential are time-, resource-, and animal-intensive. To reduce animal use and expedite evaluation, alternative in vitro approaches for compound screening have been developed but few have undergone rigorous review. FETAX measures embryonic development of the South African clawed frog, Xenopus laevis and is a test for organogenesis, which is highly conserved across amphibians and mammals. In this assay, egg batches and develop into embryos during exposure to the test substance. After 96 hr of development, individual embryos are evaluated for mortality, malformations, and growth inhibition. In 1998, the USEPA requested that ICCVAM evaluate the validation status of FETAX, its potential regulatory utility, and the critical research, development, and validation efforts needed to improve it. An Expert Panel Meeting (May 2000) deliberated on these issues (http://iccam.nih.gov) and concluded that the performance and reproducibility of FETAX was not adequate for regulatory applications and recommended that: 1) the FETAX protocol be redefined, 2) the number of endpoints be expanded to improve assay performance for identifying developmental toxicants, 3) additional,
more robust decision criteria be developed, and 4) additional validation studies be conducted. In addition, the Panel recommended that validation criteria specific for developmental toxicity, and reliable and accurate comparative databases of animal and human developmental toxicants be developed. Supported by NIHES contract N01-ES-85424 with ILS, Inc.

1221 MONOCLONAL ANTI-PHENOCYCLIDINE (PCP) IgG PRODUCES LONG-TERM PROTECTION AGAINST ADVERSE EFFECTS OF PCP
R. M. Laurencin, S. E. Ali, W. B. Gentry and S. M. Owens, Dept. of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR and Division of Neurotoxicology, National Center for Toxicological Research, Jefferson, AR.

The purpose of this study was to determine if anti-PCP IgG protects against PCP-induced effects on locomotor activity, brain weight, and brain monoamine levels. Sprague-Dawley rats (2 groups, N=4) received 18 mg/kg/day of PCP continuously for 2 weeks via a subcutaneous osmotic minipump. Once PCP reached steady-state concentrations (24 hr after implanting pumps), baseline locomotor activity was determined. One group of rats then received a control saline treatment and the other group received a single dose of anti-PCP IgG, which was 0.5 mg the molar equivalent amount of IgG in the body at steady-state. PCP-induced locomotor activity was determined immediately following the saline or IgG administration. The anti-PCP IgG caused a significant reduction in PCP-induced locomotor activity. Body weights were recorded for the entire 2 week experiment. Significant weight reductions were found in the rats receiving the saline. In stark contrast, anti-PCP IgG treated rats maintained their body weight and apparent good health. The rats were sacrificed 2 weeks after implanting SC pumps for determination of PCP concentration in 7 brain regions, tests and serum. Anti-PCP IgG significantly decreased PCP concentrations in serum, tests and all brain regions, except the brainstem. Brain regions were also examined for brain monoamine levels by HPLC/EC. For these studies, a saline control group (N=6) that did not receive PCP was included. DopAC levels were decreased in the frontal cortex of the PCP-saline group compared with the PCP-IgG group and saline control group. These results suggest that anti-PCP IgG can offer significant protective effects and improved health in the presence of continuous high dose PCP that apparently saturates anti-PCP IgG binding capacity. (Supported by NIDA Grants DA0710 and DA08 DA 339).

1222 RELATIONSHIP BETWEEN PARKINSONISM AND SERUM PROLACTIN LEVEL IN MPTP-TREATED COMMON MARMOSETS.
T. Kamensonosso, B. Lee, Y. Mori, K. Okazaki, S. Meyers, K. Fukuzaki and R. Nagaya, SNBL USA Ltd., Everett, WA.

The main neural disorder in Parkinson's disease is degeneration of dopaminergic neurons in the substantia nigra. The MPTP-treated marmoset is an established model for Parkinson's disease, since MPTP produces motor deficits characteristic of those seen in patients with Parkinson's disease. Prolactin secretion is under tonic inhibitory control by dopamine (prolactin release-inhibiting factor). We examined the relationship between Parkinsonism and apparent prolactin levels in MPTP-treated common marmoset. Male and female common marmoset (1.5-3 years old) were used for this study. Animals were treated 3 to 4 times with intravenous administration of MPTP (1.25 mg/kg) under ketamine anesthesia (with a 1 or 2 week interval between administrations). Immediately after MPTP treatment, the animals were immobilized, with flexed hind quarters, posterior tremor, and a loss of vocalization. Approximately 4 months after MPTP-treatment, in control (non-treatment) and MPTP-treated animals, spontaneous locomotor activities were measured by recordings on electromechanical counters (photocell sensor). Behaviors were observed by video recording to evaluate parkinsonian symptoms. Serum prolactin levels were analyzed using ELISA. The mean locomotor activity in all MPTP-treated animals was decreased compared with that in control animals. All MPTP-treated animals exhibited the characteristic parkinsonian symptoms such as slow and/or uncoordinated movements, however, there was individual difference in the degree of these symptoms. In the prolactin analysis, MPTP-treated animals showed higher prolactin levels compared to the control animals. The results of this study demonstrate that intravenous administration of MPTP produces motor deficits even 6 months after MPTP-treatment due to degeneration of dopaminergic neurons. This suggests that the increase in prolactin is induced by reduction of brain dopamine levels in the MPTP-treated common marmoset.

1223 CROTONONITRILE ISOMERS HAVE A DIFFERENT PROFILE OF NEUROTOXIC EFFECTS IN THE RAT.

IDPN (3, 3'-aminodipropionitrile) and allylthionine have a number of common effects on the nervous and sensory systems of the rat, including degeneration of the vestibular and auditory hair cells, cornal opacification, and gliosis in the retina and olfactory bulb. Gliosis in other specific areas of the CNS has also been observed after IDPN. The vestibular toxicity of these nitriles results in a permanent behavioral syndrome. Crotononitrile (CRO), a mixture of cis- and trans-isomers, has been demonstrated to cause both the vestibular and sensory hair cell degeneration. This study compared the effects of the two isomers of CRO, isolated to >99% purity by fractional distillation from the commercially available CRO mixture (approximately 60% cis- and 40% trans-CRO). Adult male Long-Evans rats were administered for 3 days with vehicle control, cis-CRO (80, 100, 120 mg/kg per day) or trans-CRO (250 mg/kg per day) and the changes in cornal transparency and vestibular function were assessed. Surface preparations of the vestibular sensory epithelia and the organ of Corti were observed for hair cell loss by scanning electron microscopy. Concentrations in retina, olfactory bulbs, cerebellum, striatum and cingulate cortex of GFAP; a marker for reactive gliosis, were also determined. In a dose-response manner, cis-CRO increased the rating scores in behavioral tests of vestibular dysfunction, and caused cornal opacity, hair cell loss in both the vestibular epithelia and organ of Corti, and gliosis in retina, olfactory bulbs and cortex but not in the cerebellum and striatum. This profile of neurotoxicity is similar to that shown by IDPN and allylthionine. In contrast, trans-CRO did not cause vestibular dysfunction, hair cell loss or cornal opacity; although other neurotoxic actions, currently under investigation, were evident from the motor behavior of the treated animals. We conclude that the neurotoxic effects of nitriles depend on strict structural requirements; this suggest that they may act through their interaction with specific molecular targets.

1224 PRETREATMENT WITH L-CARNITINE PREVENTS HYPOTHERMIA INDUCED BY THE MITOCHONDRIAL INHIBITOR 3-NITROPHOSPHONIC ACID (3-NPA).

An environmental toxin, 3-NPA, can cause cellular energy deficits due to irreversible inhibition of a mitochondrial Complex II enzyme, succinate dehydrogenase (SDH). We have previously shown that exposure to 3-NPA and the resulting SDH inhibition were associated with a 2°C decrease in rectal temperature 3 hrs after dosing. While pretreatment with L-carnitine (an enhancer of free fatty acid beta oxidation) prior to 3-NPA administration, prevented the oxidative stress-associated increase in the activity of free radical scavenging enzymes, it did not significantly prevent 3-NPA-induced SDH inhibition. Here, intracranial temperature was continuously measured near the cerebellum in adult male Sprague-Dawley rats injected with either 100 mg/kg, i.p. L-carnitine (LC) 60 min prior to 3-NPA (30 mg/kg, i.c.) or with 3-NPA alone. Rats were surgically implanted with temperature probes registering linear electrical responses to temperature change. Compared with baseline temperature, administration of 3-NPA alone was associated with a significant fall in temperature within 1 hr postinjection. Pretreatment with LC totally prevented this decrease (p<0.05). Data indicate that the neuroprotective effects mediated by the carnitines may result from the enhancement of energy production rather than direct or indirect interference with 3-NPA.

![Graph showing effect of L-Carnitine on brain temperature](image-url)
1225 NEUROTOXICITY PRODUCED BY DIBROMOCETIC ACID IN DRINKING WATER OF RATS.


The Safe Drinking Water Act requires that EPA consider noncancer endpoints for the assessment of adverse human health effects of disinfection byproducts (DBPs). Dibromochloroacetic acid is a known human and animal neurotoxicant, but essentially no studies have evaluated the neurotoxic potential of the brominated analog dibromocetic acid (DBDA). This study characterized the neurotoxicity of DBDA during six-month exposure in drinking water of rats, using a neurobehavioral test battery followed by perfusion fixation for neurotochemical evaluation. Adolescent male and female Fischer 344 rats were administered DBDA at 0, 0.2, 0.6, and 1.5 mg/l, producing an average intake of about 0.20, 0.72, and 161 mg/kg/day. In both sexes, weight gain was depressed in the high-dose group, but overall general health was not altered. Testing using a functional observational battery and motor activity took place before dosing began and at 1, 2, 4, and 6 months. DBDA produced dose-related neuromotor dysfunction characterized by limb weakness, mild gait abnormalities, and hypothermia, as well as sensorimotor depression, with decreased responses to a tail pinch and click. Other signs of toxicity included decreased activity and chest claspings. The neurotochemical findings was depression of myelinated nerve fibers in spinal cord white matter (lateral and ventral area) in the high-dose rats. In addition, small numbers of swollen, cosinophilic, and sometimes vacuolated neurites were observed in the spinal cord gray matter of mid- and high-dose rats. No abnormality-related changes were seen in brain, eyes, peripheral ganglia, based on the neurobehavioral data, the LOEL of this study was 20 mg/kg/day (lowest dose tested). These studies demonstrate that neurotoxicity should be considered in the overall hazard evaluation of halocarbon acids. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1226 EFFECTS OF EARLY POSTNATAL ESTRADIOL AND DIETHYLSTILBESTROL EXPOSURE ON CINCINNATI WATER MAZE PERFORMANCE OF MALE AND FEMALE RATS.

R.L. Carr, J.C. Chambers, A. Khachroo and J.R. Richardson. Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

Estrogen plays an important role in the development of the brain. Thus, exposure of developing animals to chemicals which mimic the effects of estrogen could potentially disrupt normal development leading to alteration of function including behavior. These studies were designed to determine the effect of excessive stimulation of the estrogen receptors on cognitive function using the Cincinnati water maze. In male and female rats were orally administered 72 mg/kg (I7B-estradiol (E2) or 10 mg/kg diethylstilbestrol (DES) from postnatal day 1 (PND1) through PND33. Behavioral testing was initiated using Path A (2 trials per day for 5 days with a 2 hour intertrial period). Following a 2 days non-testing period, rats were tested using the reverse path (Path B) in similar fashion. Neuronal exposure to E2 significantly reduced the number of errors made by female rats in Path A. Male rats treated with E2 also made fewer errors than controls but it was not statistically significant. The latency of the E2 treated rats to complete Path A was lower than controls but was not statistically significant. Neuronal exposure to DES resulted in an increased number of errors and longer latency in both male and female rats in Path A but these were not statistically significant with the exception of the longer latency to complete the maze by female DES rats. Neither E2 or DES treatment produced any differences in Path B errors or latency in either sex. Statistical comparison between the two daily trials in Path A demonstrated that both male and female E2 treated rats made significantly fewer errors on the second trial than the first while control rats performed similarly. This was not present in the DES treated rats. This suggests that E2 treatment during development enhances short-term memory. However, since DES did not produce similar results, it can be hypothesized that this enhancement: may not be mediated through the traditional E2 receptor.

1227 NEUROTOXICITY INDUCED BY REPEATED DOSES OF 2-DIMETHYLMALINOETHYL METHACRYLATE IN RATS.


To evaluate the neurotoxicity of 2-dimethylaminoethyl methacrylate, groups of 6 male SD rats were administered orally with 0 (corn oil), 500, or 1,000 mg/kg for 6 consecutive weeks. The following items were conducted on all rats: clinical observation (twice per day); the functional assay (once or twice per week); the histopathological examination of the central and peripheral nervous tissues. The salivation was found in the 500 and 1,000 mg/kg groups, and the tremor of the forelimbs and the clonic convolution in the upper half of the body was found in the 1,000 mg/kg group. The salivation was observed immediately after dosing and appeared from the second week. The tremor was a transient sign, occurring within 30 min after dosing, and it appeared from the fifth week. The clonic convolution was observed at the sixth week, and its trigger was the stimulation of the handling. When an attack of convolution began, the animals crumpled, thrusting out their forelimbs. Vuculation in the white matter of the spinal cord was found in all rats of the 1,000 mg/kg group. The lesions appeared symmetrically, and they were restricted in the dorsal spinocerebellar tracts. The affected nerve fibers comprised axonal fragmentation accompanying with myelin ballooning, disruption, and phagocytosis. It was considered that clonic convolution was due to the nerve fiber degeneration in the dorsal spinocerebellar tracts of the spinal cord.

1228 AVOIDANCE RESPONDING AND MOTOR ACTIVITY IN EPH A5 KNOCKOUT MICE.

L. Michon, G. D. Yancey, R. Zhou and C. W. Wagner. Department of Molecular Biology, Rutgers University, New Brunswick, NJ; Department of Pharmacology, University of Puget Sound, Tacoma, WA; Department of Biological Sciences, University of Puget Sound, Tacoma, WA; and Department of Psychology, Rutgers University, New Brunswick, NJ.

The Eph receptor family has been implicated in the regulation of axons and formation of topographic projection maps. For example, the Eph A5 receptor is expressed in differentiating neurons of the hippocampus and cortex and is thought to be involved in the development of the hippocampal and corticocortical pathways. Previous neurotoxicology analysis revealed that Eph A5 knockout mice have higher stratal dopamine and decreased caudal dopamine compared to wildtypes. Presently, male and female Eph A5 receptor knockout and wildtype mice were evaluated in the active avoidance responding paradigm and horizontal motor activity test. In the two-way active avoidance paradigm, mice were given 20 trials/day for 30 days, each consisting of a 15 sec tone CS and a 10 sec, 0.5 mA footshock US with a criterion of 18 avoidance/day. In the motor activity test, horizontal activity was recorded after 10 min in an activity monitor following baseline, saline and cocaine (0.3, 1, and 3 mg/kg). In the active avoidance paradigm, male Eph A5 knockout reached criterion by day 16, while wildtype only reached 50% by the end of 30 days. Also, female Eph A5 knockouts reached criterion by day 22 while female wildtype only reached 33% at the end of 30 days. Both male and female Eph A5 knockouts were less active following saline and all doses of cocaine. As both male and female Eph A5 knockout mice show enhanced avoidance responding and decreased activity after cocaine, these results may indicate a role for Eph A5 in the regulation of dopamine-mediated motor response. (Supported by NIH ES07148)

1229 CONFIRMATION OF INTER-OBSERVER RELIABILITY IN THE CONDUCT OF FUNCTIONAL OBSERVATION BATTERY ASSESSMENTS.


The Functional Observation Battery (FOB) is widely accepted as a sensitive tool for screening toxicology. CTBR has extensive experience performing neurotoxicity testing in accordance with GLP and stringent guidelines of the USEPA and routinely conducts of inter-observer reliability (IORs) studies to validate technical competence in the performance of FOB tests. With the advent of the ICH S7 guideline, these capabilities have been applied to CNS safety pharmacology studies of pharmaceuticals. The current study was a recent IOR assessment of 4 technicians and employed 3 positive reference compounds: amphetamine (AM), ethanol (ET) and carbachol (CA). Since the technicians were not drawn from the pool of previously trained staff, the IOR was preceded by a two-week training session. The IORs then served to validate the consistency of observations between technicians but also served to evaluate the effectiveness of the training program. The technicians were able to detect the major neurotoxic endpoints expected for each of the positive control compounds including tremors and autonomic signs with CA, ataxia and gait abnormalities with ET and bizarre stereotypical behaviors with AM. Initial variability between observers was less evident than the previous injection of the IOR assessment. Since all technicians were considered to be scoring with similar accuracy and precision, they were approved to perform FOB assessments in rats. In conclusion, the IOR performed by our laboratory provided reliable reproducible results and confirmed the effectiveness of the training program. IOR assessments provide laboratories with a means to assess the competence of their staff and Sponsors with a means to monitor the capabilities of laboratories in the performance of such tests. In order to further reduce the risk of inter-individual or temporal variability, it is critical to perform periodic IOR assessments on technical staff assigned to such studies and whenever possible ensure the same staff carry out the FOB for all occasions on a given study.
1230 DEVELOPMENT OF A MODEL FOR CHRONIC STRESS EXPOSURE IN MICE.

M. Dubovicky1, I. Benyovszky1, M. Morris2 and I. B. Luconi3.

1Pharmacology/Toxicology, Wright State University, School of Medicine, Dayton, OH; 2Inst Exp Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia; 3Inst Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia. Sponsor: P. G. Gusatad.

Chronic stress contributes to physical and mental illness and may interact with chemical toxins. Our previous experiments demonstrated that acute stress potentiates neurotoxicity (Luconi, 1999). Thus, we conducted controlled scientific studies of the response to stress preparatory to studying the interactions of stress and chemical toxins. We have developed a unique model of chronic emotional stress in mice based on intermittent shaker stress. Mice are kept in their home cages fed to a cage rack mounted on the shaker power unit. Standard pellet diet and tap water are provided ad lib. Our experimental paradigm enables the study of the neuroendocrine, cardiovascular, and behavioral responses to acute as well as chronic stress. Chronic stress did not have any unfavorable effect on the health of the mice. There was 10% of loss in body weight after 2 weeks. However, after discontinuation of chronic stress the mean values of body weight returned to the values of stress-free controls. Neuroendocrine and cardiovascular assessment revealed significant increases in plasma corticosterone, blood pressure, and heart rate in acutely and chronically stressed mice. Chronic emotional stress did not have any effect on total open field activity or on elevated plus maze behavior. However, emotional reactivity on the elevated plus maze as measured by defection rate increased in the chronically stressed mice 7 days after stress. Unlike the chronic stress, acute stress resulted in significant decreases in locomotor activity in the open field test. Our results show that the chronic intermittent shaker stress provides an excellent model for stress exposure and for use in testing stress/chemical interactions. Supported by DoD contract No. 99214005.

1231 ACUTE AND REPEATED RESTRAINT STRESS HAVE LITTLE INFLUENCE ON PYRIDOSTIGMINE TOXICITY.

X. Song1, H. Tian2, I. Brezis1, S. Proctor1 and C. Pope3. 1Physiol Sci, Oklahoma State Univ, Stillwater, OK; 2Kennedy Krieger Institute, Baltimore, MD; 3Cell Bio Lab, LSU Health Sciences Center, Shreveport, LA.

Pyridostigmine bromide (PB), a quaternary, carbamate acetylcholinesterase inhibitor, has been used to protect soldiers from possible exposure to long-term inactivators of acetylcholinesterase, i.e., organophosphorus nerve agents. Some studies suggest that stress may alter BBB integrity and allow PB to enter the brain. We evaluated the effects of acute and repeated restraint stress on PB neurotoxicity. For acute restraint stress, rats were divided into 4 groups (1) PB control (given 10 mg/kg, po), (2) PB + restraint for 2 h, (3) PB + restraint for 6 h, and (4) PB + restraint for 24 h. In all cases, rats were evaluated for cholinergic toxicity (SLID signs and involuntary movements) and sacrificed 1 hour after PB administration. Restraint stress (90 min) caused a significant increase in plasma corticosterone. Whole blood ChE was markedly inhibited (-80%) by PB whereas brain regional ChE activity (cortex, hippocampus, cerebellum) was only marginally affected (maximal 20% inhibition). PB-induced toxicity was not enhanced nor was the brain ChE inhibition increased by any stress condition. BBB integrity assessed by the accumulation of horseradish peroxidase in brain regions following intracarotid tracer injection was not increased by prior stress. Rats were also exposed to daily stress (60 min) and concurrently treated with either the non-stress or stress condition. Rats were sacrificed 4 h. PB-treated rats (both dosages) exhibited slight signs of cholinergic toxicity for the 1st day of exposure after which tolerance developed. Repeated restraint, however, had little effect on functional signs of PB toxicity. Whole blood and hippocampal ChE were markedly reduced (57-84% and 74-83%, respectively) 1 hour after the last exposure but brain regional ChE activity showed minimal and inconsistent changes. The results suggest that acute and repeated restraint stress have little effect on PB toxicity or PB entry into the brain. (Supported by grant DAMD17-96-1-0070 from US Army).

1232 ADAPTIVE RESPONSE OF BLOOD-BRAIN BARRIER DISRUPTION AND INCREASED PERMEABILITY TO PYRIDOSTIGMINE BROMIDE (PB) BY REPEATED IMMOBILIZATION STRESS.


In the present study we investigated the effects of repeated immobilization stress in male Sprague-Dawley rats on the brain penetrability of PB and its effects on the brain regional acetylcholinesterase (AChE) activity. Animals were stressed by restraint in a Plexiglas tube for 15, 30, and 60 minutes, alone or following pretreatment with 15 daily oral dose of 1.3 mg/kg PB. Brain uptake of [3H]PB (100Ci/g, oral) was used to assess the changes in the BBB permeability. Co-exposure of PB and stress resulted in significantly increased uptake of [3H]PB (~258, 170, and 64% in midbrain, cortex, and cerebellum, respectively at 30 minutes of stress) and ~256% increase in brainstem at 15 minutes of stress, as compared with exposure with PB alone. However, in all the brain regions, there was a decreasing trend in the uptake following co-exposure of PB and 60 minutes of stress. PB alone caused no significant change in AChE activity in brain regions, but significant inhibition in plasma AChE activity following treatment with PB alone. Co-exposure to midbrain AChE activity was maximally inhibited following 30 and 15 minutes of stress alone (~77 and 52%, respectively). Co-exposure to stress and PB resulted in significant inhibition in cortex, midbrain, spinal cord, cerebellum and plasma (~56-79% of control) at 30 minutes of stress whereas maximum inhibition (~56% of control) was observed in brainstem at 15 minutes of stress. Co-exposure to PB and stress for 60 minutes resulted in reduction in the extent of inhibition of AChE activity. These results suggest that short-term stress could disrupt the BBB permeability to PB, whereas prolonged exposure to stress results in adaptive response. Supported, in part by the US Army Medical Research and Material Command under contract DAMD 17-99-1-9020.

1233 VALIDATION OF A CNS SAFETY PHARMACOLOGY BATTERY IN THE MOUSE.


The ICH S7 Safety Pharmacology guidelines stipulate a core battery of tests that are required for assessment of effects on the central nervous system (CNS). In addition, follow-up studies may be required on a case-by-case basis for additional information. At CTBR, a GLP-compliant CNS battery consisting of evaluation of locomotor activity, auditory startle response, barbiturate sleep time, acetic acid writhing and anti- and pro-convulstive assays with both chemical and electrical induction of convulsions. A range of common positive control compounds with well-documented effects in these studies were used to demonstrate the effectiveness and acceptability of the assays. G6 week old male Swiss CrlCD-1/ICR were miced in all tests. Positive control compounds were formulated in saline or water and administared as a single oral gavage dose or intraperitoneal injection. Dose levels were based on published literature. Animals administered sedative or stimulant drugs and assessed for general behavioral responses, both spontaneous and forced motor activity or startle response all exhibited characteristic reactions. Extensions were seen for anesthesia time following co-administration of barbiturates and benzodiazepine or phentobarbital drugs, a reduction was seen in the number of writhes induced by acetic acid following both opioid and non-opioid analgesic administration, and escalation or diminution of the convulsive threshold was observed for both chemical and electrical induction of convulsions following alkaloid or chloralhydrate administration. All studies therefore meet their pre-defined acceptance criteria and both the core battery and follow-up studies are considered validated for use at this laboratory.

1234 A FUNCTIONAL OBSERVATIONAL BATTERY (FOB) FOR USE IN DOG TOXICITY STUDIES: DEVELOPMENT AND VALIDATION.


The most commonly used nonrodent species in regulatory systemic toxicity and safety assessment studies has been and remains the dog, with the beagle being the standard breed employed. While a standardised FOB for use in rats (based on Gad, 1982) has been incorporated into rodent studies since the late 1980's, and some work has been previously conducted towards developing a similar screen in the dog (Haggerty, 1991), progress has been limited. Given the different metabolic barrels and sensitivity of the dog compared to rodents, and the extreme desirability of having as complete a set of toxicity and/or functionality measures in the same species (to simplify and improve the accuracy of dose/response metrics), the need for such a validated methodology is clear. Study data from prior work establishes the susceptibility of dogs to a wide variety of neurotoxic agents, including 6-ANA, mevinolin, lasalocid, metronidazole, acetaminophen, organochlorine, and mercury. Additionally, the dog is likewise well established as a sensitive model for a wide range of peripheral and central nervous system active pharmacologic agents, as required by recent regulatory requirements for safety pharmacology evaluations. A robust and yet sensitive noninvasive screening methodology for detecting and pro-
viding initial quantitation and characterization of such direct and indirect neurotoxic and neuropharmacologic effects has been developed. Additionally, an analysis and interpretation component which allows differentiation of neurotoxic from neuropharmacologic activities, has been developed and validated. Comparative species and strain differences in sensitivity to neuroactive agents are also discussed, as well as means for integrating these measures into existing standard designs.

1235
CONTINUED USE OF A BEHAVIORAL TEST BATTERY TO ASSESS RHEUS MONKEY OFFSPRING EXPOSED TO COCAINE IN UTERO.

P. Morris, M. P. Gillam and M. G. Paule. Pharmacology University of North Texas, Fort Worth, TX and 2NCTR, FDA, Jefferson, AR

In order to explore possible long-term effects of gestational cocaine (COC) exposure, pregnant rhesus monkeys were treated with COC chronically from about 1 month (mo) of gestation until term (either 0 (N=3), 0.3 (N=3), 1.0 (N=3), or escalating doses up to 8.5 (N=3) mg/kg (i.m.), 3 times/day, 5 days/week). Beginning at 6 mo of age, the behavior of offspring (N=12) was monitored using an operant task battery with 4 food-reinforced tasks designed to model aspects of motivation, learning, color and position discrimination, short-term memory and attention. Between 6 and 18 mo of age, the acquisition of operant behaviors by juvenile offspring was not differentially affected by gestational COC exposure. Possible long-term effects of gestational COC exposure were examined further by measuring the sensitivity of acquired operant behaviors in young adults to acute administration of SCH23953 (SCH; 0.010, 0.010, 0.010, 0.010, 0.010, 0.010 or 0.0175 mg/kg (i.v.)), quinpirole (QN; 0.0, 0.010, 0.010, 0.050, 0.050, 0.050, 0.050, or 0.100 mg/kg (i.m.)), or apomorphine (AP; 0.0, 0.010, 0.010, 0.010, 0.030, 0.030, 0.030, 0.030 mg/kg (i.m.)). When assessed at 54 (SCH), 62 (QN), or 67 (AP) mo of age, the operant behavioral responses of offspring to acute challenges were not differentially affected by gestational COC exposure. It was concluded that gestational COC exposure had no significant effect on the subsequent acquisition of complex behavior by offspring, or the sensitivity of those behaviors to pharmacological challenge with SCH, QN or AP. Supported by NIDA IAG #224-B-99-003.

1236
NEUROLOGICAL ABNORMALITY IN WORKERS OF 1-BROMOPROPAINE FACTORY.

G. Ichihara, W. Li, E. Shinbata, X. Ding, M. Kamijima, H. Wang, Y. Liang, S. Peng, S. Itohara, Q. Fan, Y. Zhang, W. Zhong, X. Wu, W. M. Valantine and Y. Takeuchi. 1Nagoya University Graduate School of Medicine, Nagoya, Japan. 2National Evaluating Centre for the Toxicology of Fertilizer Regulating Drugs, Shanghai, China. 3Nagoya University School of Health Science, Nagoya, Japan. 4Voyz Anti-Demic and Health Station, Yongsh, China and 5Vanderbilk University Medical Center, Nashville, TN

It has been demonstrated that 1-bromopropane (1-BP), an alternative to ozone-depleting solvents, produces neurotoxicity and reproductive toxicity in rats. This study investigates the neurological effects of 1-BP in workers exposed to low concentrations of 1-BP. The survey examined 25 female workers at a 1-BP factory, 37.5 Awell (Mean 4.5 S.D.) years old, and 27 female workers from a beet factory 35.5Awell, 8 years as controls. Individual time weighted average exposure levels of the workers in the 1-BP factory were estimated using individual passive sampler tubes to be 0.32-4.99 mg/m3 median 1.00 mg/m3. Neurobehavioral and electrophysiological assessment of nerve function were performed in both groups. Vibration sense deficits were detected in the feet of 17 exposed workers but not in any of the control subjects. Distal lenticulars of the exposed workers was significantly longer than the controls, but the motor nerve conduction velocity of the tibial nerve did not show significant differences. Conduction velocities of the sural nerve and the F wave of the tibial nerve were significantly decreased in the exposed workers relative to controls. Digit Symbol, Benton Visual Retention and Pursuit Aiming test produced inferior values for the exposed group. In the Profile of Mood States testing, the exposed group exhibited lower scores than the control in Agitation, Depression, Anger, Fatigue and Confusion. These findings suggest that exposure to 1-BP at levels below or around 50 ppm in the occupational setting may affect peripheral and central nervous system function.

1237
TOXIKOGENETIC-TOXIKODYNAMIC RELATIONSHIPS IN HUMAN MEBROPAINE POISONINGS.

B. Migraignce, F. J. Baud, S. W. Borrow and C. Ramburh. Intensive Care Unit, Lariboisiere Hospital, Paris, France.

The value of PK-PD relationships in clinical pharmacology is well recognized. However, the potential interest of Toxico-kinetic-Toxidynamic (TK-TD) relationships in medical toxicology has been poorly investigated. The aim of this study was to correlate the depth of coma with plasma mebropane (MB) concentrations.

Materials and Methods: Plasma MB concentrations were measured using a colorimetric assay. The depth of coma was assessed using the Glasgow coma scale (GCS). Non-linear regression was used for modeling TK-TD relationships. Results: TK-TD relationships were studied in 7 acute MB poisonings. Three patients were previously treated with MB. Previous treatment was unknown in 2 patients. Mixed drug poisoning was noted in the 7 patients. The mean GCS at the time of hospital admission was 4+1, the mean plasma MB concentration was 1054±518 mmol/L. The mean MB concentration associated with a GCS of 3 was 714.6±277.7 mmol/L (95% CI: 546.8-882.4). The TK-TD relationship was well fitted with the sigmodal Emax model. In the 7 patients, the mean Hill coefficient (m/SD) was 6.9±4.0 (95% CI: 3.2-10.7), the mean GCS was 487.9±318.8 mmol/L (95% CI: 153.5-782.7). Two patients exhibited tolerance to the sedative effect of MB. In the 5 non-tolerant patients, the mean Hill coefficient (m/SD) was 8.0±4.4 (95% CI: 2.5-13.4), the mean GCS was 315.8±148.9 mmol/L (95% CI: 130.9-500.7). Discussion: A maximal toxic effect (GCS of 3) was associated with a wide range of plasma MB concentrations. During the course of MB poisoning, the relationship between the depth of coma and the corresponding plasma concentrations is of a sigmodal shape. The high value of the Hill coefficient in non-tolerant patients showed that a small decrease in plasma MB concentrations near the GCS was associated with a dramatic improvement in their level of consciousness. In non-tolerant patients, the mean GCS was close to the upper limit of the therapeutic plasma concentration of MB given by our toxicological laboratory ( < 200 mmol/L).

1238
EFFECTS OF PEAK CONCENTRATIONS ON THE NEUROTOXICITY OF SYNERE IN VOLUNTEERS.


The manufacture of fibreglass reinforced plastic products may give rise to substantial peak exposures to styrene. Such exposure pattern needs further consideration in terms of styrene neurotoxicity. The aim of this study was to evaluate the neurotoxic effects of short-term peak exposures in volunteers, at levels respectively the Quebec occupational exposure limits (8-h TWA: 213 mg/m3 and 15-minute average of 426 mg/m3). The volunteers had not been previously exposed to styrene and they had no documented exposure to known neurotoxicants during the study. Forty volunteers were exposed to five exposure scenarios during 6 hours: a) stable exposure to 106 mg/m3, b) variable exposure with a mean concentration of 106 mg/m3 for 15 minutes, c) stable exposure to 426 mg/m3, d) variable exposure with a mean concentration of 213 mg/m3 and 4 peaks of 426 mg/m3, and e) two stable exposures to 5 mg/m3 (control). Before and after each exposure scenario, volunteers were submitted to a battery of sensory tests (vis-sual and olfactory), neuropsychological tests (reaction time, attention, memory, psychomotoric), and self-evaluation questionnaires (mood and symptoms) in a test-retest design. The results show that the different exposure scenarios negatively influenced neither the performance to any test nor subjective signs and symptoms. Thus, the short-term exposure, below the Quebec occupational exposure limits does not induce neurotoxic effects evaluated using a battery of internationally recognized neurotoxic tests. Due caution must be exercised in extrapolation of the current results to occupational exposure situations and current exposure were used and volunteers were at rest during exposure, which exposed them to lower doses than those experienced by physically active workers. This project was supported by IRSSST, Quebec, Canada.

1239
BEHAVIOURAL EFFECTS OF PEAK EXPOSURE TO TOLUENE IN THE RAT.


Occupational exposure limits of solvents are usually based on average exposure levels during the work shift. However, recent insights recognize that the effects of inhalatory exposure to solvents may not only depend on the total dose administered but also on the exposure scenario, e.g. exposure to fluctuating vs constant concentrations. In two inhalatory exposure studies, rats were exposed to constant solvent concentrations of 1333 or 2607 ppm or to fluctuating concentrations (cycles of 1.5 h each with peaks of 8000 ppm for 15 min or 30 min) for 7.5 hours. Rats received an overall similar total dose of 10000 or 20000 ppm-hours, respectively. Visual discrimination performance was tested prior to exposure, after the 7.5 h exposure period and one day post-exposure. The different exposure scenarios induced different changes the behavioral responses of response speed, stimulus control and inhibition of responding to air-exposed rats. Slowing of response was most pronounced when measured sometime after the end of a peak exposure, the effects on stimulus control and inhibition of responding were most pronounced when measured immediately after a peak exposure or after a period of exposure to a constant concentration. In one study, spontaneous motor activity was measured continuously.
for 72 h, from one day prior to one day after the exposure-day. Different effects were observed on motor activity depending on the exposure scenario. During peak exposure, increased activity was observed (i.e., an increase and a fixed sequence that was invariant (i.e., a performance control). Following extended training, all rats received a single dose (0.6 mg/kg) of nicotine, s.c., 5-min prior to testing. Rats were then divided, based on nicotine responses, into two groups that received either two additional injections of nicotine or saline vehicle at weekly intervals. All rats then received nicotine in the fourth week. Nicotine initially decreased response accuracy and reinforcement rate regardless of the sequence (fixed or varying). Tolerance developed to the effects of nicotine in rats dosed weekly. Substantial tolerance was also obtained in rats receiving nicotine three weeks after the first dose. No differences were obtained between the two groups during sessions when nicotine was not administered. These results suggest long-term behavioral changes in nicotine sensitivity following acute nicotine administration. This abstract does not necessarily reflect USEPA policy.

1243
EFFECT OF WEIGHT MAINTENANCE AND ACTIVITY ON TISSUE LEVELS OF TRICHLOROETHYLENE IN MALE LONG-EVANS RATS.

J. E. Simmons1, W. K. Boyce1, M. V. Evans2, J. H. Rayner1, A. McDonald1, Y. M. Sey1 and P. J. Bushnell1. 1NEHEERI/ORD, USEPA, Research Triangle Park, NC; and 2RTI, Research Triangle Park, NC.

Trichloroethylene (TCE) is a common neurotoxic pollutant, contaminating air, water and food. Assessment of its risk to health will be aided by an exposure-dose-response model linking exposure to acute neurotoxicity. One test method for neurotoxicity testing used to evaluate TCE involves chronic toxicity and the use of weightmaintained animals actively working for rodent chow. Thus, part of our exposure-dose-response modeling effort is development of a physiologically-based pharmacokinetic model for TCE in weight-maintained rats. For this purpose, TCE concentrations were measured in the blood, brain, liver and fat of rats exposed by inhalation to 200 or 2000 ppm TCE for either 5, 20 or 60 min and rats exposed to TCE for 60 min and then to clean air for 60 min. Measurements were made in weight-maintained (-350 g) rats actively performing a signal detection task during inhalation exposure to TCE; weight-maintained (-350 g) rats exposed to TCE in the absence of task performance (sedentary); and, ad libitum-fed rats (-weight range, 350-450 g) that were sedentary during exposure to TCE. The observed TCE tissue concentrations were not proportional to the external exposure concentrations as a 10-fold increase in external TCE concentration (200 to 2000 ppm) resulted in greater than 10-fold increases in tissue TCE levels. As expected, due to its lipophilic nature, the highest concentrations of TCE were observed in fat. At 200 and 2000 ppm TCE, the concentrations of TCE in blood, brain, liver and fat were higher in the active, weight-maintained rats than in either the sedentary, weight-maintained rats or the sedentary, ad libitum-fed rats. Thus, activity status (active vs sedentary) appeared to be a more important determinant of the pharmacokinetic behavior of TCE than weight status (ad libitum-fed vs weight-maintained). (This abstract may not reflect EPA policy.)

1244
EXPOSURE-DOSE-RESPONSE MODELING OF THE NEUROTOXIC EFFECTS OF ORGANIC SOLVENTS.

P. J. Bushnell1, W. K. Boyce1, J. E. Simmons2, M. V. Evans2, E. M. Kenyon2, T. Levens2, W. M. Oshiro2, T. E. Sansom3, Q. T. Kranz3, J. L. Shafee1, V. E. Benignus1 and T. L. Jackson1. 1Neurotoxicology Division, USEPA, Research Triangle Park, NC; 2Experimental Toxicology Division, USEPA, Research Triangle Park, NC; and 3Department of Mathematics, University of Michigan, Ann Arbor, MI.

Risk assessments based on exposure to volatile organic compounds (VOCs) are hampered by the complexities of exposure scenarios, a lack of data regarding the mode of action of the VOCs, and uncertainties about extrapolating from animal data to human health risk. We are developing an approach to rationalize these assessments by (1) linking airborne exposure to internal dose via dosimetry modeling in rats; (2) investigating interactions of solvents with receptor systems in the CNS; and (3) comparing the relative sensitivities of rats and humans to the acute effects of VOCs. Physiologically-based pharmacokinetic (PBPK) models are being used to estimate the concentrations of VOCs in blood and brain of rats using a variety of exposure scenarios. Estimates of internal dose (concentrations of trichloroethylene or toluene in blood and brain) accurately predicted the effects of these VOCs on signal detection behavior and visual evoked potentials (VEPs). Models of action studies are underway to identify critical neurological pathways that mediate the effects of toluene on signal detection behavior and VEPs, and to test the feasibility of in vitro tests of the acute neurotoxicity of VOCs. Parallel tests of signal detection in rats and humans and inhaling toluene may yield a quantitative estimate of the relative sensitivities of the species, to facilitate extrapolation of toxicity in rats to the risk of adverse effects on human health. (This abstract does not necessarily reflect EPA policy.)
A physiologically-based pharmacokinetic (PBPK) model for inhaled toluene was developed for Long-Evan rats as a component of an exposure-dose-response (EDR) model for volatile organic compounds. The PBPK model was needed to link airborne toluene exposure to its concentration in blood and brain, the target organs for its neurotoxicity. The acute neurotoxicity of toluene was quantified in rats as impaired detection of visual signals and reduced visual evoked potentials (VEPs). The model required input from the literature for ten conditions for both of these endpoints, i.e., signal detection behavior was measured in weight-maintained, physically-active rats, whereas VEPs are measured in rats fed ad libitum and restrained during testing. Blood and brains were obtained from 64 sedentary rats, either weight-maintained (350 g) or fed ad libitum (400 - 500 g), after inhaling toluene at either 200 or 2000 ppm for 5 minutes by decontamination and downregulation. In the current study, we do not necessarily reflect EPA policy.

### 1246

**Effects of TERBUTALINE (TER) on BETA-ADRENOCETOP (BAR) SIGNALING IN THE DEVELOPING RAT: IMPLICATIONS FOR ADVERSE EFFECTS OF TOCOLYTIC THERAPY.**

J. T. Auman, F. J. Seidler and T. A. Slotkin. Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC.

Preterm labor is commonly treated with B2AR agonists, like TER, that arrest uterine contractions, but that also enter the fetus. Ordinarily, issues are protected from excessive B2AR stimulation by the Bar/BAR antagonists, with a greater effect in the liver. Despite the reduction in cardiac BARs, there was no sensitization of cell signaling mediated through adenyl cyclase (AC). Using stimuli that operate at different loci in the AC cascade, we found that TER treatment on P 2-5 did not desensitize any of the signaling proteins: treatment on P 11-14 actually increased the activity of the G-protein and AC G-prot. Similarly, in the liver, heterologous sensitization of AC signaling occurs nearly all of the BAR downregulation, so that BAR-mediated AMP generation was maintained. Our results indicate that the developing organism does not show agonist-induced desensitization of the BAR/AC signaling pathway and can actually show sensitization in response to TER treatment. In light of the critical role played by cAMP in cell replication and differentiation, these unique responses may contribute to cardiac anomalies and alterations in glucose handling noted in infants whose mothers received tocolytic therapy. Finally, numerous environmental and hormonal factors converge on the generation of cAMP, so that sensitization of the AC pathway by cAMP may render these individuals more vulnerable to developmental disruptions. (Supported by USPHS HD09713)

### 1247

**ENHANCED SKIN CARCINOGENICITY IN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR β-NULL MICE.**

D. J. Kim, F. J. Gonzalez and J. M. Peters. Veterinary Science and Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University College of Medicine, University Park, PA; and Laboratory of Metabolism, NCI, Bethesda, MD.

The peroxisome proliferator activated receptor-β (PPARβ) shows high levels of expression in the skin, although the role of this receptor in skin function is uncertain. Interestingly, the epidermal hyperplasia and response induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) is greatly increased in PPARβ-null mice. This suggests that PPARβ functions to attenuate epidermal cell proliferation in response to TPA. In this study, PPARβ-null and wild-type mice were subjected to the two-stage chemical carcinogenesis bioassay to elucidate a functional role of PPARβ in skin cancer. PPARβ-null mice were more sensitive to TPA than wild-type controls as evidenced by differences in skin morphology after two-three topical applications of TPA. This phenomenon continued for the duration of the six-month experiment. The formation of papillomas in PPARβ-null mice was greatly accelerated compared to wild-type mice and the percentage of PPARβ-null mice that developed papillomas was significantly greater than wild-type controls. After 24 weeks of TPA application, the average number and size of papillomas in PPARβ-null mice were significantly greater than wild-type mice. The results strongly suggest that PPARβ null-cell proliferation. In the absence of a functional PPARβ, skin tumor formation and growth are greatly enhanced in this two-stage chemical carcinogenesis model, which suggests that activation of this receptor could inhibit skin cancer. Additionally, these data suggest that humans with a polymorphism or mutation in PPARβ gene may be at increased risk for developing skin cancer. (Supported by ROI CA86007, J.M.P.)

### 1248

**MOLECULAR AND BIOCHEMICAL ALTERATIONS IN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR β (PPARβ) NULL MOUSE SKIN IN RESPONSE TO 12-O-TETRADECANOPYLPHORBOL-13-ACETATE (TPA).**

F. S. Harman, S. Yin, D. J. Kim, E. J. Gonzalez and J. M. Pettey. Veterinary Science and Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University College of Medicine, University Park, PA; and Laboratory of Metabolism, NCI, Bethesda, MD.

The biological roles of PPARβ remain largely unknown. The use of the PPARβ-null mouse model should greatly increase our understanding of the receptor function in target tissues. Recent evidence suggests a role for PPARβ in epithelial cells. In the absence of PPARβ, epidermal hyperplasia is greatly enhanced in response to TPA suggesting that PPARβ may function to attenuate cell proliferation. Potential PPARβ target genes that could regulate the cell cycle have not been identified. Using microarray analysis, several genes were identified as being regulated by PPARβ. Northern blot analysis confirmed that expression of these genes in response to TPA is mediated by PPARβ. The specific role of these target genes in epithelial cell cycle control is currently under investigation. In addition to molecular alterations, biochemical changes in intracellular phosphorylation events were also examined, since this type of regulation is a common mechanism found in replicating cells. Significant differences in phosphorylated proteins were found between wild-type and PPARβ-null mouse skin in response to TPA. Specifically, cytosolic levels of phosphorylated mitogen-activated protein kinase (MAPK) and several protein kinase C (PKC) isoforms were approximately 2-fold higher in null mouse skin treated with TPA compared to wild-type controls. Additionally, western blot analysis revealed that the translocation of PKC from the cytosol to the particulate fraction is markedly different between TPA-treated PPARβ-null and wild-type skin. Combined, these results suggest that PPARβ regulates epidermal target gene expression that ultimately leads to modulation of phosphorylation events, which in turn regulate cell cycle progression. (Supported by ROI CA86007, J.M.P.)

### 1249

**DIFFERENTIAL EFFECTS OF 3'-METHOXY-4'-NITROFLAVONE (3'-M4'NF) ON INDUCTION OF LUCIFERASE AND CYP1A1 IN RECOMBINANT MOUSE HEPATOMA CELLS.**

J. Zhou and T. A. Gazievic. Toxicology, University of Rochester, Rochester, NY.

Aryl hydrocarbon receptor (AhR) is a transcription factor belonging to the BHLH-PAS gene superfamily. Ligand-free receptor resides in cytosol as a complex associated with several other proteins. Upon ligand activation, the receptor undergoes a series of transformation processes that involve translocation to the nucleus, association with AhR translocation partner (Arnt), and interaction with dioxin responsive element (DRE), leading to the enhancement of gene expression such as CYP1A1. Cell lines stably transfected with AhR-responsive, mouse CYP1A1 DRE-driven luciferase (2DLuc) are sensitive bioassay systems for the study of AhR signaling mechanisms and determining the presence of dioxin-like environmental contaminants. Although studies have shown that TCDD induces 2DLuc and CYP1A1 in a similar pattern, there is little information about the induction of these two genes by structurally different AhR ligands. It is possible that differential ligands may elicit a distinct AhR conformation, which may lead to distinct expression profiles between 2DLuc and CYP1A1 (or other genes). To test the hypothesis, the recombinant cells were treated with 3'-M4'NF. The induction of 2DLuc and CYP1A1 was examined by three different endpoints: mRNA transcription, protein expression and enzyme activity assay. The studies demonstrated that 3'-M4'NF produces a differential effect on the induction of reporter gene and CYP1A1. 3'-M4'NF has no or little activity in enhancing the expression of 2DLuc by itself but is able to antagonize TCDD-induced reporter gene induction completely, an indication of a "puckering" effect. In contrast, it promoted the CYP1A1 expression in a dose-dependent manner, an indication of partial agonist. The results presented herein provide us further insights and qualifications for the use of reporter genes to study the AhR signaling pathway. (Supported by NIH grant ES07072 and Center grant ES01247)
1250

2D GEL ELECTROPHORESIS, MUTATION ANALYSIS, AND SEQUENCE/STRUCTURE-BASED PHOSPHORYLATION PREDICTION OF ARYL HYDROCARBON RECEPTOR (AhR) TYROSINE RESIDUES SUGGEST PHOSPHORYLATION AT TYROSINE 9.

G. D. Minavage, D. Vorobjkina and T. G. Gazievic. Toxology Training Program, Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY.

The AhR is a ligand-activated member of the bHLH transcription factor family. Xenobiotics such as TCDD bind to the AhR to mediate pleotrophic toxicities. For the AhR, a phosphoprotein with demonstrated charge heterogeneity, DNA binding is a critical step necessary to modulate target genes that mediate TCDD toxicity. We previously reported that treatment of AhR/Arnt complexes with tyrosine (Y) phosphatase or mutation of AhR Y9 to phenylalanine (AhRYP9) abrogates DNA binding in vitro. We hypothesized that utilization of computational analysis together with 2D gel electrophoresis (2-DE) of AhR Y mutants would correlate AhR charge heterogeneity with predicted Y phosphorylation and indicate a possible role for AhR Y phosphorylation in DNA binding. An N-terminal truncated mutant (AhR1-399) was shown to retain DNA binding potential in vitro. Point mutations of each of the twelve Y-terminal residues indicated that only the Y9 mutant abrogates DNA binding, suggesting a specific functional role for this conserved amino acid. Computational sequence and structure-based prediction analysis indicated that Y9 also maintains conserved phosphorylation potential across all species sequenced. To corroborate this, a truncated AhR WT and point mutants were analyzed by 2-DE. These data indicate more basic isoforms (AhR9Y9F and other sites with a high predicted phosphorylation potential (AhR9Y372F) while sites with low predicted phosphorylation potential (AhR9Y65F) have isoforms similar to AhR WT. These data suggest consistency between charge heterogeneity and the predicted phosphorylation potential of AhR Y residues and putative phosphorylation at AhR Y9. This, along with previous data, suggests that Y9 phosphorylation plays a role in AhR DNA binding. (Supported by NIH Grant ES02513, Training Grant ES07086, and Center Grant ES01247.)

1251

COMPREHENSIVE ANALYSIS OF GENE EXPRESSION IN HEPATOPA CELLS EXPOSED TO THE PEROXISOME PROLIFERATOR WY, 14, 643: EVIDENCE OF AN AUTO/REGULATORY PATHWAY.

K. A. Burnt, D. Kreoler, B. Beida, C. Nugent, M. Taylor and J. Vanden Heuck. Center for Molecular Toxicology and Carcinogenesis, Penn State University, University Park, PA and Roche BioScience, Toxicology and Neurobiology Unit, Palo Alto, CA.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor family. PPARs are activated by a large group of chemicals that include industrial pollutants which are called peroxisome proliferators (PPs). PPs are believed to cause cancer in rodents by altering gene expression and affecting the phenotype of the target cell. Experimental WY gene expression microarrays from hepatoma cells treated with the PP WY14, 643 showed the expected increase in mRNA for fatty acid catalase genes including acyl-CoA oxidase, fatty acid binding protein and cytochrome P450 4A1. A wide variety of lipid metabolism, cell cycle and growth regulatory genes were also observed to be PPs-responsive. The transcription microarray experiments also revealed a variety of protein kinases, phosphatases, and signaling molecules as being PP-responsive. Several of these proteins have been shown to affect PPARα activity as well as being key players in the cell cycle. For example, members of the MAPK, JAK/STAT and PKA pathways were affected by PPs at the mRNA, protein and activity level. Inhibition of these kinases may result in an alteration in PPARα activity. In addition, we tested the hypothesis that PPs ability to regulate kinase cascades may affect the phosphorylation of important cell cycle regulators. Of particular interest is Retinoblastoma (Rb). Following treatment with PPs, an increase in Rb phosphorylation at serines 807 and 811 is observed. We are studying the mechanism by which this important protein is being affected by PPs. The data from this study can also be used to speculate on other genetic pathways that affect nuclear receptor and also highlight awareness of the importance of cross-talk between PPAR and growth factor signaling. (Funded by NIH ES07799 and DK49099.)

1252

EFFECTS OF CYTOSOLIC CONVERSION OF ESTRONE TO ESTRADIOL ON RAINBOW TROUT ER BINDING AFFINITY.

M. A. Tapper, R. Kalanszky, J. Denny, T. Henry and P. Schmieder. USEPA, Mid-Continent Ecology Division, Duluth, MN and USEPA, NHEERL, Experimental Toxicology Division, Research Triangle Park, NC.

The relative binding affinity (RBA) for estrone (E1) to the rainbow trout (Oncorhyncus mykiss) estrogen receptor (rER) was measured as part of a larger effort to determine chemical structural features predictive of chemical estrogenicity in fish. Estrone RBA was found to vary considerably (14 to 68%) among cytosolic preparation more than expected due to experimental variability. The cytosolic concentrations of E1 after 20h incubation at 4°C (binding assay conditions) was subsequently found varying from 32 to 89% of the original E1 concentration, raising concerns over possible differences in hydroxysteroid dehydrogenase levels in individual fish. In mammals, 17β-hydroxysteroid dehydrogenases (HSD) are responsible for the interconversion of estrogens and androgens to and from active and inactive forms. HSD 1 has been identified as the primary enzyme responsible for the conversion of E1 to 17β-estradiol (E2). Mammalian HSD 1, localized in ovary and placenta, is present at low levels in mammalian liver. Knowledge about HSD activity in fish but likely a HSD 1-like enzyme in liver cytosols preparations converts E1 to E2. Depending on the cytosol used, 15 to 68% of the initial E1 was converted to E2. No conversion was detected in water or cytosol buffer under the same conditions. A positive correlation was noted between increasing cytosolic E2 levels and RBA presumably attributed to binding 2. Little is known about HSD activity in fish but likely a HSD 1-like enzyme in liver cytosols was used under the same conditions. Therefore it appears that measurable HSD-like activity in fish cytosolic preparations can readily alter steroid concentrations, and lost apparent RBA measurements, and that the RBA of rER for E1 is closer to 14% than 68%. This abstract does not necessarily reflect EPA policy.

1253

CHARACTERIZATION OF ARYL HYDROCARBON SIGNALING PROTEINS FROM XENOPUS LAEVIS.


Embryos of the African clawed frog, Xenopus laevis, are used in FETAX (frog embryo toxicology and xenobiotics) to assess developmental toxicity of chemicals. Halogenated aromatic hydrocarbons (HAHs) interact with the Aryl hydrocarbon receptor (AhR), a lipid-activated membrane receptor, and can be potent developmental toxicants. However, several frog species are relatively resistant to TCDD toxicity. Although assessments of TCDD toxicity in X. laevis vary, some reports suggest that this species may also be relatively insensitive. In other vertebrates, TCDD toxicity is mediated by the aryl hydrocarbon receptor (AhR), a lipid-activated transcription factor, and by ARNT (aryl hydrocarbon receptor nuclear translocator), the dimeric partner of AhR. To investigate the mechanisms of TCDD sensitivity and resistance in X. laevis, we have isolated cDNA sequences encoding AhR and ARNT from 96 hour embryos. X. laevis resembles many fish in the expression of two distinct AhR genes. However, phylogenetic analysis demonstrates that both X. laevis AHRs are orthologs of mammalian AHR1; neither resembles the AhR2 genes common to fish. The two proteins, AHR16 (833 a 94.1 kDa) and AHR1B (833 a 93.4 kDa), share 80% amino acid identity, suggesting that they evolved rather recently, perhaps coincident with the tetraploidization of the Xenopus genome approximately 30 mya. X. laevis represents the first species in which multiple AHR1 genes have been observed. Analysis of the single X. laevis ARNT sequence detected revealed that it is an ARNT2 ortholog. ARNT2 is widely expressed in certain fish, but is restricted to kidney and CNS in mammals. Since sequencing multiple X. laevis ARNT clones detected only ARNT2, we suggest that it is the predominant ortholog expressed at this life stage. FETAX is used as a model for predicting the developmental toxicity of materials to other vertebrates. An understanding of the molecular mechanisms underlying sensitivity differences to halogenated aromatic hydrocarbons should contribute to the development of FETAX as a model for developmental toxicity of samples containing these compounds. [NIH: ES11130]

1254

COMPARATIVE STUDY OF HUMAN AND MOUSE AH RECEPTOR FUNCTION USING CHIMERIC RECEPTORS.

P. Ramadoss and G. H. Peredew. Graduate Program in Biochemistry, Microbiology and Molecular Biology and Department of Veterinary Sciences and the Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA.

The aryl hydrocarbon receptor (AhR) is a ligand induced transcription factor that exists in the cytoplasm complexed with hsp90 and XAP2. The most widely studied AhR ligand is TCDD. The intra- and inter-species differences in sensitivity to TCDD may occur as a result of differences in receptor structure. Sequence variations in the mouse and human receptors resulting in quantitatively different responses to TCDD are of importance since rodent models are used in vivo toxicity studies. Two chimeras of human and mouse AhR were generated in order to study functional differences due to the low homology in the C-terminal half of the AhR. A unique BamH1 restriction site in both receptors at amino acid residue 424 was used to remove and exchange the C-terminal halves of the receptors. Constructs expressing both FLAG- and yellow fluorescent protein-tagged chimeras were generated. Cellular localization studies in COS-1 cells showed that mouse
1255  DIOXIN ALTERS PROLIFERATION, DIFFERENTIATION AND SENESCENCE IN KERATINOCYTES.
S. Ray, Dept. of Molecular and Biomedical Pharmacology, University of Kentucky Medical Center, Lexington, KY. Sponsor: H. W. Swann.
TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin), a prototype of the polyaromatic hydrocarbons, elicits a variety of toxic, teratogenic and carcinogenic responses in both animals and humans. Many effects of TCDD are mediated by the aryl hydrocarbon receptor (AhR), a BHLH/PAS transcription factor. Ligand activation of AhR alters both cellular proliferation and differentiation of keratinocytes, but the signaling pathways involved in these processes are poorly understood. The objective of this study was to delineate the mechanisms involved in dioxin tumor promoting activities using normal human keratinocytes (NHK). Using western blot analysis of involucrin and flanking epidermal differentiation markers, we have shown that TCDD decreases differentiation of NHKs. This result is supported by flow cytometric analysis, which show that TCDD decreases the number of differentiating keratinocytes while increasing the number of non-differentiating keratinocytes. On performing cell cycle analysis using propidium iodide, we found that treatments with TCDD increases in the number of cells in S-phase and decreases the cells in G1/G0 phase of the cell cycle. By riboprobe analysis we found that the decrease in differentiation is accompanied by a significant decrease in the expression of p16INK4a, p53, Rb, p73β and p14ARF. Some of these results were also verified by western blot analysis. p16INK4a is a cyclin-dependent kinase inhibitor that regulates the G1 to S progression and increases both differentiation and senescence. Hence, to determine the effect of TCDD on senescence we performed senescence-associated β-galactosidase staining and found that TCDD causes a decrease in senescence of keratinocytes. These results suggest that TCDD may act as a tumor promoter by inhibiting both differentiation and senescence and a decrease in the expression levels of p16INK4a. Overall, these results suggest that the tumor-promoting activities of TCDD is in part mediated by down regulation of these important cell cycle regulatory proteins.

1256  GENERATION OF TEMPERATURE-SENSITIVE LIVER CELL LINES FROM PPAR-REDEFICIENT AND WILD-TYPE C57B1/6 MICE.
J. P. Gray, E. S. Tien, N. M. Pezic and J. P. Vanden Heuvel. Center for Molecular Toxicology, Pennsylvania State University, University Park, PA.
Several novel cell lines were generated from the neonatal liver cells of either wild-type (wt) or PPAR-Re-deficient (null) C57B1/6 mice using a temperature sensitive SV40 virus. These cells were passaged at the virus's permissive temperature (34°C) and gene expression experiments are performed at the non-permissive (37°C) temperature. The wt and null cell lines express equivalent concentrations of albumin as well as acyl-CoA oxidase (ACO) and fatty acid binding protein (FABP) mRNA, indicating the retention of liver-specific genes. PPARα presence or absence in the wild type or PPARα-null lines was confirmed by genotype PCR as well as western blotting. The cell lines also retain the ability to appropriately respond to peroxisome proliferators whereby Wy-14, 643 (50μM) treatment for 6 hours failed to induce ACO in the null cells, but significantly increased expression in wt cells. Cell cycle analysis also confirms a defect in the response of PPARα-deficient cells to Wy-14, 643 treatment. These lines will help us to investigate the mechanistic aspects of PPARα-dependent signaling in response to peroxisome proliferator treatment and effectively recapitulates what has been observed in vivo in the mouse models. (Funded by NIH ES07799 and DK49009).

1257  THE EFFECTS OF GREEN TEA EXTRACTS AND INDIVIDUAL TEA CATECHINS ON AHF FUNCTION.
Previous investigations have implicated green tea as an effective method for promoting the inhibition of polycyclic aromatic hydrocarbon-induced cancers in animal models of chemical carcinogenesis. In an effort to understand the mechanism and compound's response to these compounds, the effects of green tea extracts (GTE) and individual catechins on the function of the aryl hydrocarbon receptor (AhR) were determined. Green tea (GT) was organically grown and subsequently fractionated by TLC. The chemical composition of these fractions, as well as a commercially available GTE, were determined by NMR. All GT fractions inhibited tetrachlorodibenzo-p-dioxin (TCDD)-induced transcription of a dioxin-responsive element (DRE)-dependent luciferase reporter in stably transfected mouse hepatoma cells in a concentration dependent manner. The most potent of these was then examined for its ability to compete with TCDD for binding to the AhR, and to inhibit TCDD-induced binding of the AhR to DREs in vitro. From these studies, it was shown that GTE blocked TCDD-induced binding of the AhR to DNA. However, it did not compete directly with TCDD for binding to the receptor. To determine the green tea component(s) responsible for the observed effects, individual catechins were tested in these systems and found to be capable of competing with TCDD for binding to the AhR. This data suggests that individual catechins are capable of altering AhR transcriptional activity and that these compounds may function through a mechanism unlike typical AhR antagonists. (Supported in part by NIH grant ES07026, and Center Grant ES01247).

1258  STRIKING VARIABILITY IN THE STRUCTURES OF ARNT AND ARNT2 IN THE RAT.
M. Korkalainen, T. Liimatta and R. Puhjanvirta. Department of Environmental Health, National Public Health Institute, Kuopio, Finland. Department of Public Health and General Practice, University of Kuopio, Kuopio, Finland. Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland and National Veterinary and Food Research Institute, Kuopio Departments, Kuopio, Finland.
The biological and toxic effects of dioxins are largely mediated by the aryl hydrocarbon receptor (AhR). The AhR functions as a ligand-activated transcription factor binding DNA as a heterodimeric complex with a structurally related bHLH/PAS protein, the AhR nuclear translocator (ARNT) or its homologue in the brain, ARNT2. We recently discovered 3 splice variants of ARNT in an extremely TCDD-resistant rat strain, Han/Wistar (Kuopio), which appear to account for its resistance. In ARNT protein, so far only one splice variant (deletion of exon 5) has been described in the literature. To find out whether structural deviations of ARNT could be related to TCDD sensitivity, we cloned hepatic ARNT and hypothalamic ARNT2 cDNAs by blunt-end cloning after RT-PCR from untreated H/W and TCDD-sensitive Long-Evans (Turku AB) rats. To our surprise, we recorded several different products of alternative splicing of ARNT mRNA: in addition to exon 5, deletions occurred at 3' end of exon 6 (NTs 354-394) and at 5' end of exon 11 (NTs 964-978). A small insertion (IC) was detected at the 5' end of exon 20. Interindividual variation transpired in the number of GCA repeats at the 5' end of exon 16. In the case of ARNT2, an insertion of 31 NTs appeared at NT 2128. Importantly, this insertion contained a premature stop codon resulting in a truncated protein at the C-terminus, with potentially a repression-type function. All other variants represent in-frame changes, but at least the two larger deletions are likely to seriously impair protein function. There were no major differences between the two rat strains in relative expression levels of the variants as assessed by semi-quantitative RT-PCR implying that they do not contribute to strain-specific sensitivity to TCDD.

1259  THE ARYL HYDROCARBON RECEPTOR (AhR) HOMOLOGUE OF THE SOFT SHELL CLAM (M. ARRAEINA) DOES NOT BIND PROTOTYPICAL AHR BINDING GANDS TCDD OR BNF.
The aryl hydrocarbon receptor (AhR) mediates various toxic effects of polycyclic aromatic hydrocarbon compounds, including 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). Some populations of softshell clams (M. arenaria) have historically exhibited a high prevalence of gonadal neoplasia. Although the etiology of these lesions is unknown, their incidence is correlated with environmental herbicide (TCDD-contaminated) usage. One hypothesis proposes an AhR-mediated pathway of tumorigenesis. To investigate this, a clam AhR homologue (cDNA) was cloned. Amino acid alignments indicate domains characteristic of vertebrate AHRs. Despite these structural similarities, binding analyses using in vitro expressed clam AhR or clam cytosolic proteins showed that this AhR homologue...
1260
HORMONAL ACTIVATION OF LACTATE
DEHYDROGENASE GENE EXPRESSION IN BREAST
CANCER CELLS.
X. Li and S. H. Safe. *Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

In previous studies, we identified the 92- to 37-region of the lactate dehydrogenase (LDH) gene promoter as estrogen (E2)-responsive in transient transfection studies in MCF-7 human breast cancer cells. This promoter sequence contains a CAMP response element (CRE) and nuclear extracts from MCF-7 cells bound [32P]–P86-36 (CRE-sequence from LDH promoter) in gel mobility shift assays to form a broad retarded band which was decreased in intensity after competition with an unlabeled consensus CRE oligonucleotide. The retarded band was supershifted after incubation of nuclear extracts with antibodies that bound ATP-1 and CREB. Constitutively active protein kinase A and an inducer of CAMP (cholera toxin) induced reporter gene activity in breast cancer cells transfected with the E2-responsive plD1II construct (contains the -56 to -9 LDH gene promoter insert) confirming that CAMP/PKA induction also activates the CRE. Hormonal activation of plD1II and related constructs was also inhibited by cotransfection with KREB, a dominant negative form of CREB. Activation of CREB and ATF-1 by E2 through non-genomic pathways was confirmed in studies showing that 10 nM E2 caused a 7- and 27-fold induction of reporter gene activity MCF-7 cells transfected with GAL4-ATF1 and GAL4-CREB reporter constructs and a construct containing five tandem GAL4 response elements. Thus, LDH upregulation by E2 is due to non-genomic activation of the CAMP-PKA pathway. (Supported by NIH ES09253 and ES09106)

1261
2, 3, 7, 8-TETRACHLORIDIBENZO-P-DIOXIN
(TCDD) INDUCES PLASMINOGEN ACTIVATOR
INHIBITOR-1 THROUGH AN ARYL HYDROCARBON
RECEPTOR-MEDIATED PATHWAY IN A MOUSE
HEPATOMA CELL LINE.
D. S. Son* and K. K. Reiman1,2. *Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS and 1Section of Environmental Toxicology, GSF-Institut fur Toxikologie, Neuherberg, Germany.

2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), an ubiquitous environmental pollutant, elicits a variety of toxicities and is a well-known carcinogen. TCDD alters the expression of many genes including CYP1A1/2, CYP1B1, glutathione S-transferase Ya, aldehyde dehydrogenase, NAD(P)H:quione oxidoreductase, TGF-alpha, TGF-beta, plasminogen activator inhibitor-2 and interleukin-1beta. The present study investigated the effect of TCDD on plasminogen activator inhibitor-1 (PAI-1) in a mouse hepatoma cell line (HePac1c7). Based on Western and Northern blots, TCDD induced dose- (0, 0.1, 1 & 10 nM) and time- (0, 3, 6, 12, 24 & 48h) dependent PAI-1 in Hepa1c7 cells. However, TCDD did not induce PAI-1 in the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (Arnt)-deficient mutants derived from Hepa1c7 cells, indicating a functional role of the AhR/Arnt complex in this effect. Transfection with PAI-1 promoter resulted in increased PAI-1 promoter activity in Hepa1c7 cells treated with TCDD, but no such effect occurred in the Arnt-deficient cell lines, implicating involvement of the AhR and Arnt proteins. In addition, alpha-naphthalene and phenanthrene, two AhR antagonists, blocked the enhancing effect of TCDD on PAI-1 promoter coupled luciferase activity in Hepa1c7 cells. The results revealed that the induction of PAI-1 by TCDD occurs in the same dose range as other known TCDD-induced AhR-mediated signal transductions, implicating PAI-1 as the most recently found gene induced by TCDD.

1262
HYPOXIA INDUCES PROTEASOME-DEPENDENT
DEGRADATION OF ESTROGEN RECEPTOR 6 (ERo)
IN HUMAN BREAST CANCER CELLS.
M. Stoner and S. H. Safe. *Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

Cell growth under hypoxic conditions induces a cascade of responses linked to changes in enzymes responsible for metabolic adaptation for growth and survival under low oxygen conditions. Hypoxic conditions in tumors can lead to a more clinically aggressive phenotype and, therefore, we have investigated the effects of reduced oxygen levels on key regulatory genes/proteins in estrogen receptor α (ERo), protein levels of estrogen receptor α (ERo) in human breast cancer cells. ZR-75 cells were maintained in an atmosphere of 21% (normoxia) or 1% (hypoxia) oxygen in the presence of 0-900 μM cobaltous chloride to approximate hypoxic conditions. Western blot analysis of whole cell lysates from ZR-75 cells grown under normoxia or hypoxia for up to 24 hr showed that levels of several nuclear transcription factors, including Sp1, Sp3, hypoxia inducible factor 1B (HIF1B or Arnt) and the aryl hydrocarbon receptor (AhR), were relatively unchanged. In contrast, HIF1α protein was not detected under normoxic conditions, but accumulated to very high levels between 3-24 hr after exposure of cells to 500 μM cobaltous chloride or 1% O2 treatment. This increase in HIF1α was due to inhibition of proteosome-dependent degradation of HIF1α under normoxic conditions. In contrast, growth of ZR-75 cells in 500 μM cobaltous chloride or 1% oxygen resulted in >70% degradation of ERα protein and, in time course studies, this response was observed after growth for >3 hr in hypoxic conditions. Treatment with the proteasome inhibitor MG132 did not affect levels of ERα protein in cells maintained in 21% oxygen, whereas hypoxia-induced degradation of ERα protein was reversed by the proteasome inhibitor. The role of estrogen and antiestrogen on ERα protein and hormone-induced transactivation is complex and dependent, in part, on "optimal" expression of ERα protein. (Supported by NIH ES04176 and ES09106)

1263
NOVEL MECHANISM OF TCDD TOXICITY:
SEQUESTRATION OF ARNT FROM AN ENDONGENOUS
ROLE IN P53 GENE INDUCTION.
M. S. Hoagland, J. Yang and H. L. Swanson. *Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY.*

Most of the toxic and carcinogenic actions of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a ubiquitous environmental contaminant, are mediated by its ability to bind and activate the aryl hydrocarbon receptor (AhR). The physiological effects of TCDD exposure include altered drug metabolism, disruption of endocrine signaling and tissue-specific alteration of cellular processes including proliferation, differentiation, and apoptosis. Indeed, AhR directly mediates the gene induction of several xenobiotic-metabolizing enzymes via interacting with its DNA binding partner, ARNT; however, the mechanisms by which AhR mediates many of the TCDD effects is poorly understood. One mechanism by which the AhR may mediate TCDD toxicity includes the sequestration of ARNT from an endogenous role. ARNT is a promiscuous nuclear transcription factor that not only homodimerizes but also dimers to transcription factors of the hypoxia signaling pathway (HIF-1α) and neurogenin (Sim). To investigate the permissive role of ARNT in modulating other signaling pathways, we performed transient transfection assays using a reporter gene regulated by the P53 promoter and c-myc and ARNT expression plasmids. In addition to our discovery that ARNT is able to enhance c-myc induction of the P53 gene, we have also discovered that this occurs independent of DNA binding by ARNT. In another approach, we have analyzed endogenous P53 mRNA levels within stably transfected MCF7 cells that over-expressed ARNT, c-myc or both. Our data indicates that TCDD toxicity may be mediated by activation of AhR, which sequester ARNT, impairs the induction of P53, and accelerates cell cycle progression and/or inhibits apoptosis.

1264
INTERACTION OF AH RECEPTOR WITH PHENOLIC
ANTIOXIDANT SIGNAL TRANSDUCTION.
Q. Mai, K. L. Kunzer1, H. Burdeste1 and M. Denison2. 1HELD/MTBB, CDNCNOS, Morgantown, WV and 2Dept. of Environmental Toxicology, University of California, Davis, CA.

The aryl hydrocarbon receptor (AhR) mediates a spectrum of adaptive and toxic responses to the environmental contaminant TCDD and related halogenated aromatic hydrocarbons. AhR may also play a role(s) in development, growth, and differentiation of tissues in the absence of an exogenous ligand. The broad range of TCDD toxicity and AhR function suggest that the mechanism of AhR action involves multiple signaling mechanisms. In this study, we examined the interaction of AhR with phenolic antioxidant signal transduction, which involves oxidative signaling, Phenolic antioxidants, such as tert-butyldihydroquinone (tBHQ), hydroquinone (HQ), or catechin, induce the expression of CYP1A1 in mouse Hepa1c17 cells; the induction is enhanced by inhibition of protein synthesis by cycloheximide (CHX) (stermed superinduction). Induction by the antioxidants is both concentration and time-dependent. Furthermore, phenolic antioxidants activate the transcription of AhR and Arnt, as they are absent in AhR or Arnt defective variant cells, and are mediated through the DRE-containing enhancer of CYP1A1, as they are reconstituted in a CYP1A1 enhancer-luciferase reporter expression system. These findings demonstrate that phenolic antioxidants can activate AhR-mediated gene transcription and suggest interaction of AhR with antioxidant mediated oxidative signaling.
into reporter plasmids. A 230-bp responsive fragment, localized approximately N3.5 kb upstream of the transcriptional start site, was required for PXR-mediated induction as examined in transient transfection experiments. In addition, quantitative autoradiographic analysis of VP16-PXR stimulated enhancer-dependent reporter gene activity, producing a 10-fold induction of luciferase activity, thus confirming that the PXR-distal UGT1AI responsive sequence function is functional. Taken together, these observations demonstrate a regulatory mechanism for UGT1AI induction that is dependent upon nuclear receptor nuclear RXR, through PXR may provide a defensive mechanism against the toxicity of endogenous and foreign chemicals. (Supported in part by USPHS grant GM49135).

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LIGAND-INDEPENDENT PHYSIOLOGICAL MECHANISMS FOR AHR ACTIVATION.

Y. C. Cho, Pharmacology, University of Wisconsin, Madison, WI; Sponsor: C. Letofsky.

The aryl hydrocarbon receptor is a ligand-activated basic helix-loop-helix (BHLH)/Per-Arnt-Sim domain transcription factor. Although the mechanism of AHR activation by xenobiotics is well understood, activation of AHR in the absence of exogenous ligand remains unclear. Here we have demonstrated an alternate regulatory mechanism for AHR activation in the absence of exogenous ligand in both suspended and monolayer cells using mouse embryonic fibroblast (MEF) cell lines which contain integrated mouse Cyp1b1 promoter constructs linked to luciferase reporters responding to AHR activation. First, the AHR activity was increased in mulluscule-suspending fibroblasts as effectively as by TCDD in confluent monolayer. Second, overlay of monolayer fibroblasts with mulluscule substantially increased AHR activity while retaining adhesion. Suppression stimulated AHR activity was completely insensitive to an antagonist, a-naphthoflavone (aNF) indicating that activation is not due to a constitutive ligand. Suspension activation was selectively inhibited by Ro 32-8220, a PXR inhibitor. AHR activation of UGT1AI at a sarcoma cell line. We project that these inhibitors target the suspension activation of AHR. Several specific cellular inhibitors affect each process similarly (Thapsigargin, MG132, and geldanamycin). These data characterize a ligand-independent physiological process for AHR activation.

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GENETIC EVIDENCE THAT THE P23 CO-CHAPERONE REGULATES HUMAN DIOXIN RECEPTOR SIGNALING IN A YEAST MODEL SYSTEM.

M. B. Cox and C. A. Miller, Environmental Health Sciences, Tulane University Health Science Center, New Orleans, LA.

The dioxin (or aryl hydrocarbon) receptor is a nuclear receptor that binds toxic ligands (e.g., TCDD) and it requires the action of Hsp90 and cofactors for proper function. The dioxin receptor exists in a cytoplasmic complex which contains a dimer of Hsp90, other co-chaperone proteins, and p23 prior to ligand exposure. Whether p23 has a role in dioxin receptor signaling is not known, in vitro studies suggest that the p23 co-chaperone protein enhances the stability of dioxin receptor-Hsp90 complexes following ligand binding. This stabilization may involve p23 ATP-dependent interaction with the nucleotide-binding site of Hsp90. We used a yeast genetic model system that expressed functional human dioxin receptor and Arnt proteins to test whether p23 affects dioxin receptor signaling. Deletion of the SBA1 gene (the yeast p23 homolog) reduced ligand-mediated dioxin receptor signaling in a reporter gene assay and reduced ligand potency by approximately fivefold. Signaling and ligand potency were restored to wild type levels when SBA1 was expressed in this strain. More importantly, human p23 efficiently substituted for yeast p23 in this assay. To further investigate the role of p23 in dioxin receptor signaling, we used a temperature sensitive Hsp90 mutant strain with a severe dioxin receptor signaling defect both at the permissive and non-permissive temperatures. The mutation in this hsp90 strain maps to the ATP2/p23 binding pocket, suggesting that the dioxin receptor signaling defect and temperature sensitivity may be due to impaired p23-Hsp90 interactions. In support of this hypothesis, we found that SBA1 or human p23 overexpression suppressed temperature sensitivity and the dioxin receptor signaling defect in this mutant. These results provide the first genetic evidence that p23 mediates dioxin receptor signaling and suggest that p23 levels may be a determinant of ligand-mediated toxicity by TCDD and related compounds.

1270

FUNCTIONAL ANALYSIS OF AH RECEPTORS CONTAINING MUTATIONS WITHIN THE NUCLEAR LOCALIZATION SIGNAL (NLS).

Z. Song and R. S. Polett, Biology, University of South Florida, Tampa, FL.

The nuclear localization signal (NLS) of the Ah receptor (AhR) spans amino acids 12-39 and overlaps the basic region that is involved in DNA binding. Studies were initiated to evaluate the importance of the NLS in nuclear localization, AhR degradation, and AhR-mediated gene induction. Stable cell lines were generated in the
Hepa-1 cell line that expressed 1) wild type AHR (wtAHR), 2) AHR containing a K to A substitution at amino acid 13 (NLS-1) and 3) AHR containing an R to A substitution at amino acid 12 and a K to A substitution at amino acid 13 (NLS-2). Expression of NLS-1 or NLS-2 in vitro revealed that both proteins could bind to DNA in a TCDD-dependent manner to the same level as wtAHR. Immunohistochemical analysis of the NLS-1 and NLS-2 stable cell lines showed that the mutated AHRs were predominantly cytoplasmic and did not translocate to the nucleus in the presence of TCDD. However, the staining intensity of the NLS-1 and NLS-2 became reduced following exposure to TCDD for 4-16 hours. These results were confirmed by Western blot analysis of various AHR mutants and showed that NLS-1 was reduced by 60-80% following 16hr TCDD exposure while NLS-2 was reduced by approximately 50% after 16hr TCDD exposure. Interestingly, while neither NLS-1 nor NLS-2 could be detected in the nucleus, cells expressing these proteins exhibited TCDD-mediated CYP1A1 induction that was 10-20% of that observed in cells expressing wtAHR or in the wild type Hepa-1 line. Thus, these results support the hypothesis that AHR can be proteolytically degraded within the cytoplasmic compartment, but suggest that the NLS-1 and NLS-2 mutations are not absolute in blocking nuclear localization. Supported by ES10591.

1271 ANALYSIS OF RAINBOW TROUT AH RECEPTOR ISOFORMS (RTAHR2) IN CELL CULTURE REVEALS CONSERVATION OF FUNCTION.
K. Marks-Saige, B. Necoita, and R. S. Pellegrini. Biochemistry Medical University of South Carolina, Charleston, SC and Biology, University of South Florida, Tampa, FL.

Two distinct aryl hydrocarbon receptor (AHR) cDNAs have been isolated from rainbow trout. These proteins represent rTAHR2a and rTAHR2b are 57% identical at the amino acid level but are reported to have distinct functions with regard to AHR-mediated gene regulation when expressed in COS cells. To evaluate this hypothesis, these proteins were functionally evaluated in vitro and in the Chinese hamster lung cell line, E36. To facilitate the ability to detect and measure the expression of these proteins, both rTAHR2 isoforms were tagged with the FLAG peptide on the carboxy-terminus. When the rTAHR2 proteins were expressed in vitro and then incubated with 2, 3, 7, 8-tetrachlorodibenz-p-dioxin (TCDD) in the presence of either mammalian or rainbow trout ARNT, they failed to form functional complexes that could associate with xenobiotic response elements (XRE) in a ligand-dependent manner. Use of different temperatures, protein concentrations or extracts from trout cells did not affect the negative results. In contrast, both proteins exhibited positive function on AHR-mediated signaling when expressed in E36 cells. Both rTAHR2 isoforms showed a cytoplasmic distribution in the uninduced state and could drive expression of a reporter gene under control of the trout CYP1A3 promoter. Although both proteins could induce reporter gene activity to the same magnitude, the EC50 values of the two isoforms for TCDD differed by an order of magnitude with the rTAHR2b isoform less responsive to TCDD. When function of the rTAHR2 isoforms were tested in the context of the dominant negative rARNT, isoform, TCDD-mediated induction of reporter gene activity was significantly reduced compared to when rARNT, was silenced. Thus, both rTAHR2 isoforms appear to exhibit positive function in AHR-mediated signaling suggesting conservation. Supported by ES 08980.

1272 A SINGLE AMINO ACID DIFFERENCE UNDERLYS THE DIFFERENTIAL TRANSMISSION ACTIVITIES OF THE RAINBOW TROUT ARYL HYDROCARBON RECEPTORS (RTAHR2a and RTAHR2b).
F. A. Anderson, R. L. Tanguay, W. Heideman, and R. E. Peterson. Environmental Toxicology Center, University of Wisconsin, Madison, WI; School of Pharmacy, University of Wisconsin, Madison, WI; and Pharmacological Sciences, University of Colorado Health Sciences Center, Denver, CO.

Two rainbow trout aryl hydrocarbon receptor cDNAs (rTAHR2a and rTAHR2b) were recently isolated and characterized. The predicted amino sequences share 98% amino acid identity yet their functional properties differ. Both rTAHR2a and rTAHR2b bind 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), dimerize with rainbow trout ARNTs (rARNTb), and recognize dioxin response elements in vitro. However, in a transient transfection assay in COS-7 cells the proteins show differential activity towards a reporter driven by the rainbow trout cytochrome P450 promoter. In response to 10 nM TCDD, cells co-transfected with rARNTb and rTAHR2a showed an 8.6-fold increase in luciferase activity whereas cells co-transfected with rARNTb and rTAHR2b showed only a 1.5-fold increase. Our objective was to determine which of the amino acid substitutions are responsible for the differences in rTAHR2a and rTAHR2b transactivation activity. Chimeric rTAHR2s were constructed by switching sequences between the two rTAHR2s and an N-terminal region was responsible for the differential transactivation activity of the proteins. To determine the specific residues underlying the differential activity we utilized site-directed mutagenesis to convert certain rTAHR2 amino acids to those found in rTAHR2a.

When amino acid 111 in rTAHR2b, located just N-terminal to the PAS domain, was changed from lysine to glutamate, transactivation activity of the altered rTAHR2b was similar to that of NPCs. In these substitutions are made in the zebrafish and human AHRs at this residue, activity is similarly altered indicating that this amino acid is essential for the structure and function of the aryl hydrocarbon receptors.

1273 DO DIFFERENCES IN THE DEER MOUSE AH RECEPTOR LIGAND BINDING DOMAIN EXPLAIN WHY DDE IS A CYPIA1 INDUCER IN THIS SPECIES?
R. L. Dickerson, D. Setachan and L. T. Frame. Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX.

Previously, we reported that DDE exposure in the deer mouse induces ethoxyresoruvin-deethylase activity and that this activity is neutralized by rat-CYP1A1 antibody but not rat-CYP1B1 antibody. This was accompanied by an increase in CYP1A1 message as determined by RT-PCR. In contrast, exposure of laboratory rat and mouse to DDT or DDE results in CYP2B induction, a phenomenon not observed in the deer mouse, a New World species. Induction of CYP1A1 by environmental chemicals predominantly involves binding of the compound to the Ah receptor ARNT, and binding of the complex to dioxin response elements in the 5' Flanking region of the CYP1A1 gene. DDE has been shown to bind with the murine Ah receptor. One hypothesis for the ability of DDE to induce CYP1A1 in the deer mouse is that the deer mouse Ah receptor differs significantly in the ligand binding domain from the laboratory mouse. To test this hypothesis, we isolated cDNA from deer mouse liver, produced Ah receptor and ARNT cDNA by RT-PCR, and sequenced the PCR products. The most significant difference in the Ah receptor was in position 236 in which the deer mouse has a valine and the house mouse a methionine. Other differences were in position 306 (valine to isoleucine), 317 (lysine to arginine), 329 (methionine to isoleucine) and 331 (tyrosine to histidine). The deer mouse Ah receptor binding domain also differed from the rat in positions 236, 306, 316 and 331. We are currently in the process of determining the effect of each of these substitutions on xenobiotic binding.

1274 MOUSE HEPATOCYTE RESPONSE TO PEROXISOME PROLIFERATORS: DEPENDENCY ON HEPATIC NONPARENCYHAL CELLS AND PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR α (PPARα).

Peroxisome proliferators (PPs) are rodent nongenotoxic hepatocarcinogens that induce a range of proliferative and differentiation responses and suppress apoptosis in rodent hepatocytes. PPs act through the PP activated receptor α (PPARα). Recent studies of PPARα have implicated tumour necrosis factor (TNFα) and hepatic nonparenchymal cells (NPCs), the major source of TNFα in the liver, have also been implicated in mediating the rodent hepatocytic response to PPs. We have used an established in vitro model to investigate the interactions between PPARα and NPCs in regulating the response to PPs. Using normal hepatocyte cultures containing around 20% NPCs, the PPARα agonist (5μM) induced DNA synthesis and suppressed TGFB1 induced apoptosis. However, when the NPCs were removed by differential centrifugation, nafenopin did not induce DNA synthesis nor suppress apoptosis in the pure hepatocytes. Repopulation of the normal hepatocyte cultures by mixing together the pure hepatocytes and the previously separated NPCs in the same proportion as in the original cell preparation (17.7 ± 8.7% NPCs) restored these responses to nafenopin. Interestingly, nafenopin was still able to induce β-oxidation in the pure hepatocyte cultures, consistent with NPCs being required for PPARα-induced growth but not for proliferative proliferation. Next, we evaluated the role of NPCs in the hepatocytic dependency upon NPCs. Interestingly, NPCs isolated from PPARα null mice, like those isolated from the wild type NPCs, restored the hepatocytic response to nafenopin. However, as expected, PPARα null hepatocytes remained non-responsive to PPs, irrespective of the genotype of the added NPCs. These data support a role for NPCs in facilitating a response of hepatocytes to PPs that is ultimately dependent on the presence of PPARα in the hepatocyte.

1275 DOWNREGULATION OF LACTOFERRIN BY PPARα LIGANDS ASSOCIATED WITH THE RODENT HEPATOCARCINOGENICITY OF PEROXISOME PROLIFERATORS.

In rats and mice, peroxisome proliferators (PPs) cause liver cancer, associated with suppression of apoptosis and increased hepatocyte replication. PPARα (PP activated receptor α) is a ligand-activated transcription factor that mediates the growth
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HEPATIC EFFECTS OF PPARα ARE DISTINCT FROM PPARγ


The PPAR receptor family includes three receptor subtypes PPARα, PPARγ, and PPARδ. The subtypes are all members of the steroid/hydroxysteroid nuclear receptor superfamily of transcription factors. PPARα is abundant in rodent liver and plays a role in regulation of fatty acid metabolism. PPARα is rapidly expressed in most tissues, including liver, but its biological function is unclear. PPARα agonists have been reported to lower lipids in animal models, however, it is not known if this effect is distinct from activation of PPARγ. We used a potent and selective PPARα agonist (GW501516) to compare the effects of activation of PPARα to those induced with PPARγ agonists (GW7647 and fenofibrate). In in vivo assays, high concentrations of GW501516 cross-react with PPARα receptors. Four days of oral administration of low and moderate doses of GW501516 to Wistar Han rats did not cause peroxisome proliferation (PP), but PP occurred in rats that received a high dose. We hypothesized that induction of PP induced by GW501516 in rodents was due to activation of PPARα consistent with liver levels of GW501516 at the high dose. Gene expression analyses support this hypothesis in that peroxisomal bifunctional enzyme mRNA levels were increased in only the rats that received the high dose of GW501516 and in rats that received both PPARα agonists. Serum triglycerides decreased in all treated groups with the largest change occurring in rats that received the high dose of GW501516 and both PPARα agonists. Lipid lowering was seen at doses below those that resulted in the induction of PP. Increases in liver weight were seen in rats given the high dose of fenofibrate; although, a dose-related increase of hypertriglyceridaemia and granular eosinophilia was seen in the livers from GW501516- and fenofibrate-treated rats. Results suggest that PP is caused by activation of PPARα and that the effects of GW501516 on lipid lowering are independent of the induction of PP.

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PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR ALPHA IS ESSENTIAL FOR LIVER REGENERATION FOLLOWING PARTIAL HEPATECTOMY.

M. D. Wheeler, O. M. Smutny, L. Rugs and B. G. Thurman. Pharmacology, University of North Carolina, Chapel Hill, NC.

Liver regeneration following partial hepatectomy involves several signaling mechanisms including activation of the small GTPases Ras and RhoA. 1, 2, in response to mutations 3, leading to DNA synthesis and cell proliferation. Peroxisome proliferator activated receptor alpha (PPARα) regulates the expression of several key enzymes in lipoprotein metabolism. The role of PPARα in cell proliferation was tested. Peroxisome proliferator activated receptor alpha (PPARα) regulates genes, namely pyrophosphate synthase and HMG-CoA synthase and HMG-CoA synthase, which are significantly increased in wild-type but not in PPARα-/− mice. Also, under these conditions, membrane-association of Ras and RhoA was high in wild-type mice but was not increased in PPARα-/− mice. Concomitantly, Ras and RhoA were elevated in the cytosol in PPARα-/− mice. Deletion of PPARα blunted the degradation of cell cycle inhibitors p21 and p27kip1, prevented activation of cdk2 and cdk4, and blunted phosphorylation of Rb protein. These results support the hypothesis that PPARα is necessary for cell cycle progression in regenerating mouse liver via mechanisms involving Ras and RhoA.

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THE COMMON MARMOSET (CALLITHRIX JACCHUS) IN DRUG SAFETY STUDIES.


Callithricids are not adapted to captivity, except the common marmoset, for which a database is now available in toxicological, reproduction, and regulatory submissions. Animals of defined health status, age and history are available since purpose-bred colonies are established. We have defined background data on clinical pathology, electrocardiography, ophthalmology and histopathology facilitating interpretation of toxicology studies. Marmosets are the smallest non-rodents used in toxicology and have therefore reduced dietary and particularly substance requirements. They can thus be employed early in the drug development process. Their small size was not always an advantage because of limited amounts of blood which can be taken on studies, and because of difficulties in performing some examinations (e.g. ophthalmology). Blood analysis is now more easily possible with microtechniques, except for toxicokinetics. In this case satellite animals are recommended in order to not jeopardize results. The eye is small, deep-seated and eyelids are difficult to maintain open, making fundus photographs difficult, but possible for experienced ophthalmologists. Marmosets are not yet used routinely in reproductive toxicity, although presenting several advantages: rapid maturation, short pregnancy, and multiple births. The main disadvantage consists of the absence of external signs of the ovarian cycle preventing to detect gestation early enough without repeated blood withdrawals. Timing of evaluation and pregnancy can however be determined by monitoring urinary or fecal hormones. In addition, similarity to human primates suggests that the marmoset should be a good experimental model, suitable for teratological investigations. Several reproductive biology studies have been performed. Unfortunately, a large database is not yet available for reproductive toxicology studies. Of the use of the marmoset could be more often envisaged, for example to possibly replace the cynomolgus monkey in reproductive toxicity, a larger database could be built and, as a consequence, this species would be more easily accepted as an alternative.

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A COMPARISON OF SELECTED LABORATORY INVESTIGATION PARAMETERS IN CYNOMOLGUS MONKEYS FROM MAURITIUS AND CHINA.


The limited availability of primates for preclinical safety evaluation studies is a continuing issue. Huntingdon Life Sciences has accumulated background data from animals supplied from Mauritius over many years, but more recently other sources have been investigated. Yunnan in China has been identified as a suitable source and a number of projects have been completed with these animals. The purpose of this review is to compare results for the parameters normally investigated in the laboratory investigations conducted during preclinical toxicity tests: hematomterology, blood chemistry and urinalysis. In a recent study the two sources. While review of blood chemistry and urinalysis data from animals from the two sources indicated few consistent differences in any of the standard parameters included in safety evaluation studies, there were some marked differences seen in the haematology parameters. This was most apparent in erythrocyte values. The potential for differences in haemoglobin parameters in cynomolgus monkeys from different sources is a possible confounding factor in the interpretation of the results of toxicological studies. It is concluded that the source of cynomolgus monkeys must be taken into account in the interpretation of haematological data and in fact, ideally, cynomolgus monkeys from the same source should be used throughout a programme of work on the same compound.

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EFFECTS OF A SINGLE INTRAMUSCULAR INJECTION OF KETAMINE IN CYNOMOLGUS MONKEYS.


Ketamine is frequently used to produce temporary chemical restraint in primates for procedures performed during toxicological studies. The cynomolgus monkey is a common species used on these studies. However, limited information is available on the acute effects of ketamine administration on the various parameters evaluated during toxicity studies. Effects of ketamine administration on parameters typically evaluated on these studies could confound the interpretation of the data.
Thus, the effects of ketamine were evaluated in cynomolgus monkeys. Following IM administration of 10 mg/kg of ketamine or 0.9% saline, monkeys were evaluated for clinical effects including onset and recovery from sedation as well as food intake. Clinical pathology parameters including hematology, coagulation, platelet aggregation, serum chemistry and urinalysis/urine chemistry were also determined at multiple time points. Sedation occurred within 2 to 4 min following ketamine administration and lasted 20 to 33 min. Clinical effects of ataxia, hypotreactivity and sitting posture were present up to 2 hrs post-administration. Monkeys were fully recovered by 4 hrs post-administration. Food intake was decreased on the day of ketamine administration. There were no ketamine-related effects on hematology, coagulation and platelet aggregation parameters measured at 10 min, 24 hrs and 4 and 9 days post-administration. A minimal decrease in serum potassium concentrations (3.2-3.5 mmol/L, 26% decrease of 3.8-5.2 mmol/L) occurred in all ketamine administered monkeys at 10 min post-administration. Urine pH was minimally to mildly low (6.0-7.5, 22% decrease of 7.5-8.5) in 24 hrs post-administration compared to control. These data indicate that a 10 mg/kg dose of ketamine is suitable for temporary chemical restraint of cynomolgus monkeys. However, evaluation of clinical pathology endpoints should be initiated no earlier than 24 hrs post-ketamine administration based on the minimal limited changes that occurred within 24 hrs of ketamine administration.

1281 INFRARED THERMOMETRY FOR REMOTE BODY TEMPERATURE MEASUREMENT IN THE CYNOMOLGUS MONKEY.


Infrared thermometry is commonly used in toxicity studies for the determination of body temperature in nonhuman primates. This approach necessitates capture and handling of the animal. We, therefore, investigated the suitability of remote infrared thermometry for surface body temperature measurement in an established nonhuman primate toxicology model, the Cynomolgus monkey (Macaca fascicularis). Infrared (Gesthermálmas) and infrared (Raynger RMX84PD, Raytek) determinations were performed in eight male and female animals. Infrared measurements were conducted on caged animals and were recorded for the forehead and, among male animals, also for the testis. For all animals, data were collected for both techniques twice daily (8 am and 12 am) on 9 occasions at least one day apart. Baseline body temperature by the rectal approach in male and female animals was 38.7±0.5 °C. Forehead temperature was 31.6±0.8 °C in male and female animals. Testis surface temperature was 33.5±0.7 °C and was similar at 8 am and 12 am. Results obtained for male and female animals were comparable for both techniques. Also, the data measured were nearly identical for the 9 occasions and the two timepoints during the day. Our observations suggest that infrared temperature assessment is not suitable for determination of core body temperature. However, infrared measurements were surprisingly stable over daytime and over several days. Hence, the infrared approach is suitable for monitoring body temperature in surface temperature without the need of capture and handling of experimental animals.

1282 CIRCADIAN RHYTHM OF ACTH AND SERUM CORTISOL IN CONSCIOUS MONKEYS USING AN AUTOMATIC SAMPLING METHOD.

R. Nagas, A. Akune, N. Horai and G. Kito, Shin Nippon Biomedical Laboratories (SNBL), Ltd., Kagoshima, Japan.

In animals, severe stress from restraint for blood sampling can cause marked changes in biological parameters such as hormone levels. In this study, ACTH and serum cortisol levels were measured in blood sampled from conscious, unrestrained monkeys using an automatic blood sampling apparatus, to determine if the sampling method affected circadian variations. Methods: Eight male cynomolgus monkeys (BW 4 - 6 kg) were used. A polyurethane catheter for blood sampling was aseptically inserted into the femoral vein under anesthesia, and connected to a fluid swivel by a stainless steel. The swivel was connected to an automatic blood sampling apparatus by a polyurethane tube. Blood (1.5 mL) was automatically drawn every four hours and stored in a chamber (4°C). Samples were assayed for ACTH and serum cortisol levels by radioimmunoassay. Results and conclusion: Blood was sampled at starting three times after surgery for catheter insertion. ACTH and serum cortisol showed stable values from four weeks after surgery. ACTH levels were 13 - 18 pg/mL (daytime) and 7 - 10 pg/mL (nighttime) from four weeks after surgery, while serum cortisol levels were approximately 20 µg/dL (daytime) and 10 - 20 µg/dL (nighttime). Circadian rhythms were observed in both ACTH and serum cortisol levels, with high values during the day and low values at night. Furthermore, serum cortisol levels increased markedly according to the load of ACTH (5 µg/kg). These results demonstrate that the measurement of stable and exact ACTH and serum cortisol concentrations in monkeys is possible using an automatic blood sampling apparatus.

1283 A MODEL OF CISPLATIN-INDUCED RENAL IMPAIRMENT IN THE CYNOMOLGUS MONKEY.


Preclinical toxicity studies for human protein drug candidates are generally conducted in normal, healthy non-human primates, which may not be representative of the target patient population. Many individuals in the target patient population may have a compromised organ system due to the existing disease-state or previous drug exposure, such as chemotherapy. While the drug candidate alone may not induce a particular toxicity, it could potentially exacerbate a pre-existing injury. For this reason, a non-human primate model of renal impairment can be a valuable tool to evaluate potential exacerbation of pre-existing renal injury by the drug candidate. This study was conducted to establish a model of mild renal-impairment in the cynomolgus monkey using clinically relevant doses of the highly nephrotoxic chemotherapeutic agent cisplatin. Adult male monkeys were administered 35 or 58 mg/m² cisplatin on Days 1 and 22 via intravenous infusion, and all animals were evaluated for changes in clinical signs, body weight, and clinical pathology indices. In order to characterize the time course of cisplatin-induced nephrotoxicity, serum chemistry parameters were evaluated at various timepoints throughout the study and two animals from each dose group were euthanized on Days 7, 21, and 42 for microscopic analysis of kidney tissue. Mild elevations in serum BUN and creatinine levels were observed in 1/6 animals in the low-dose group, whereas moderate to marked elevations were noted in 3/6 animals in the high-dose group. While renal tubular necrosis ranging from minimal to marked was noted in both animals in both dose groups at all timepoints, the incidence and severity of the renal injury was greater in the high-dose group. This study demonstrates that administration of clinically relevant doses of cisplatin results in renal toxicity in the monkey and that the nephrotoxic effects of cisplatin vary within each dose level, with a higher incidence and more severe changes occurring at the higher dose.

1284 THE USE OF THE MINI-PIG IN BIOMEDICAL RESEARCH.

R. J. Hadland, Scantox A/S, Lille Skensved, Denmark.

The popularity of the mini-pig in pharmacokinetic and safety evaluation studies has increased rapidly over recent years. A better appreciation of the similarities between man and the mini-pig together with increased concerns over the use of non-human primates are two of the principal reasons for this increase. To ensure that other researchers are aware of the potential advantages of this species presentation of information is essential. Summary data relating to frequently measured variables including electrocardiogram, clinical pathology and organ weights will be presented. The use of the mini-pig in the development of dermal products, and in the investigation of reproduction toxicity will be illustrated with data from completed studies. In addition, a number of disease models have been developed to specifically address the use of the mini-pig in Parkinson's disease, atherosclerosis and diabetes research. Data from completed studies showing the induction of symptoms of Parkinson's disease following the administration of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and the successful use of this species in this type of research will be described.
1286 DEVELOPMENT AND VALIDATION OF A CANINE FUNCTIONAL OBSERVATIONAL BATTERY (FOB) FOR USE IN TOXICITY STUDIES.

J. A. Wisniewski, J. C. White, F. E. Newton, R. J. Briscoe and T. J. Baird. Pharmacology, MPI Research, Mattawan, MI.

Systematic observational evaluations for potential test article related acute and chronic neurobehavioral effects are often standard components of general toxicity studies conducted on newly-developed pharmacologic and chemical entities, such as food additives, therapeutic drugs, and industrial/agricultural agents. The beagle dog is a frequently employed non-rodent species in regulatory toxicology studies, and in a considerable amount of discovery/efficacy work. However, the vast majority of standardized neurobehavioral evaluations are performed in rodents (Iovin, 1968; Gad, 1982), and progress towards a validated FOB in canines (i.e., Haggerty, 1991) has been slow. Prior investigations have established the susceptibility of dogs, like rodents, to a variety of neurotoxic agents. Still more literature establishes the dog as a sensitive model for detecting and characterizing the adverse neurofunctional effects of pharmaceuticals. The need for, and utility of, a robust and yet sensitive screening methodology for detecting and providing initial characterization of such neurotoxic and neurofunctional effects is clear, and has now been developed and validated in the beagle dog. Initial validation of this 39-item FOB was performed using four common pharmacologic positive control compounds: haloperidol (3.0 mg/kg, p.o.), pilocarpine (2.0 mg/kg, s.c.) and amphetamine (2.0 mg/kg, s.c.). Results indicate that this observational battery is sensitive to the known acute pharmacological effects of these agents. Additional work is planned to further establish the capacity of this FOB to detect and characterize neurobehavioral pathology associated with chronic exposure to known neurotoxins.

1287 PERFORMANCE VALIDATION AND DEMONSTRATION OF THE SENSITIVITY TO DETECT DRUG INDUCED ALTERATIONS USING A LOCOTOR ACTIVITY SYSTEM FOR LARGE ANIMALS.


Neurotoxicity regulatory guidelines require that automated systems which quantify activity be evaluated to ensure the reliability of operation across devices and that the system be able to detect chemically induced changes in experimental endpoints. This study was designed to evaluate the performance and sensitivity of a system (Hamilton-Kinder MotorMonitor Activity System) to measure locomotor activity in large animals. The performance validation consisted of eight sessions for each of four enclosures; two each day, one in the am and one in the pm for four consecutive days. At random intervals throughout the 1-hour session a remote control was used to stir an object in each enclosure. The total rest time of each performance session for the object was calculated manually and compared to the total rest time recorded by the system software. To demonstrate the sensitivity of the system to detect drug-induced changes in motor activity, 12 adult male Beagles were used in a Latin Square design in which each animal served as its own saliner control and received first 0.2-mg olanzapine (0.1, 0.3 and 2.0 mg/kg, i.m.) and then clozapine (1.3 and 10 mg/kg, i.m.) during the course of the study. Basic movements (fine + ambulatory counts) were recorded in each 60-minute test session which consisted of twelve 5-minute epochs. The results for the performance evaluation indicated a mean difference of 2 seconds between the manual and software calculated total test times for each enclosure over four consecutive days, which met the acceptance criteria of ± 5 seconds. Administration of olanzapine resulted in detectable dose-related increases of 50%, 66% and 79% in basic movements above control values, while clozapine induced detectable dose-dependent decreases of 29%, 59% and 79% below basal movements. These results demonstrate that the performance of this activity system is reliable and can detect chemically induced changes in locomotor activity for Beagles.

1288 CONFIRMATION OF BLOOD IN FECES USING HEMATEST® REAGENT TABLETS: COMPARATIVE EVALUATION WITH CANINES, NON-HUMAN-PRIMATES, AND RATS.


Hematest® Reagent Tablets, marketed for the detection of occult blood in human feces, have been used to confirm the presence of blood in laboratory animal fecal material. Discrepancies between initial observations and assay results forced into question the utility of the test in laboratory animals. The purpose of this study was to determine whether Hematest® Reagent Tablets provide useful information for confirming the presence of blood in laboratory animal feces. Fecal observations were recorded from 5 canines and non-human primates (NHP) per sex and from 5 cages of rats per sex (2 per cage) for 10 days. Fecal samples for each animal or cage were tested per day with the Hematest® Reagent Tablets. All fecal samples were visually observed for the presence of red pigment and those containing red pigment were tested in lieu of a random sample. Of the 396 canine samples collected, approximately 4% were visually noted to contain red pigment; 73% of those samples observed with red pigment tested positive for blood in feces. Of the total number of samples observed with or without red pigment, 61% tested positive for the presence of blood with Hematest® tablets. In NHP, 8% of all fecal samples observed contained red pigment, of which 91% subsequently tested positive for blood in feces. Hematest® results were positive in 46% of the total NHP fecal samples collected. In rats, no samples observed during the 10 days were visually noted to contain red pigment; however, 80% of all fecal samples collected tested positive for occult blood. These findings indicate that in the species examined and manner used, Hematest® Reagent Tablet assay results do not agree with the observations of red pigment (or lack thereof) in feces. Because of the high incidence of positive results in naive animals, the test may be inappropriate and misleading when used in laboratory animals to confirm a visual observation of red pigment as blood in feces.

1289 FECAL BILE ACID EXCRETION AND PROFILE IN THE BEAGLE DOG CHARACTERIZATION ACCORDING TO SEX AND AGE.


Beagle dogs are commonly used as a nonclinical model for the safety assessment of industrial chemicals, pesticides and human pharmaceuticals. In general each laboratory maintains a historical data base for routine parameters including hematology and clinical chemistry values. However, similar data are limited or not available for non-routine tests such as fecal bile acid (FBA) excretion and profile. Thus, as a reference, FBA excretion and profile were determined in male and female beagle dogs at ages ranging from 6 to 18 months. Feces were collected over a 48-hour period and were processed and evaluated enzymatically for there were visual concentrations of 3α-hydroxy bile acids (which account for approximately 85% of total bile acid levels in dogs). These samples were further analyzed by LC/MS to provide concentrations of specific α-hydroxy bile acids -cholic acid (cholic), deoxycholic acid (deoxy), chenodeoxycholic acid (cheno), and lithocholic acid (litho). The results obtained are presented in the table below. Fecal bile acid excretion decreased with age in males. Deoxycholic acid was the predominant bile acid in feces. There were no major changes in the proportions of the major α-hydroxy bile acids between sexes and among the different age groups. However, the ratio of Deoxycholic-Cholic acid was greater in females than males indicating greater intestinal bacterial dehydroxylation in females.

<table>
<thead>
<tr>
<th>SEX</th>
<th>Age (months)</th>
<th>Fecal Bile Acids (molecules/ke/day)</th>
<th>Cholic (%)</th>
<th>Deoxy (%)</th>
<th>Cheno (%)</th>
<th>Litho (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE</td>
<td>6</td>
<td>45.0</td>
<td>56</td>
<td>76</td>
<td>2.4</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>30.8</td>
<td>9.6</td>
<td>75.1</td>
<td>2.3</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>19.9</td>
<td>7.6</td>
<td>73.5</td>
<td>2.2</td>
<td>16.7</td>
</tr>
<tr>
<td>FEMALE</td>
<td>6</td>
<td>40.3</td>
<td>6.5</td>
<td>84.8</td>
<td>1.6</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>36.7</td>
<td>9.7</td>
<td>78.3</td>
<td>1.7</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>29.0</td>
<td>4.5</td>
<td>81.3</td>
<td>1.3</td>
<td>12.9</td>
</tr>
</tbody>
</table>

*The proportion or percentage of each of the bile acids was calculated by dividing the concentration of each bile acid by the total concentration of the bile acids examined.
A COMPARISON OF FECAL BILE ACID EXCRETIONS IN CD, WISTAR, LONG EVANS, HSD-SD, AND F344 FEMALE RATS.


Rodent suppliers maintain and publish hematologic and clinical chemistry values for routine parameters. However, similar data are not available for non-routine tests such as fecal bile acid excretion. To provide this information for reference, and to compare strain variations, fecal bile acid concentrations were determined for five different strains of female rats: CD:CD(SD)BR and CD:ICR(BCR (Charles River, Portage, MI), C57L(LE)BR (Charles River, Raleigh, NC), and Hsd:SD and F344/N Hsd (Harlan Sprague-Dawley, Indianapolis, IN). The age of the females ranged from 8 to 11 weeks. Feces were collected over a 72-hour period and were processed and evaluated enzymatically for the concentrations of 3α-hydroxy bile acids (which account for approximately 70% of total fecal bile acids). These samples were further analyzed by HPLC to determine the concentrations of specific bile acids: cholic acid, chenodeoxycholic acid, deoxycholic acid, chenodeoxycholic acid (chen), and lithocholic acid (litho). The results obtained are presented in the table below. Fecal bile acid excretions ranged from 67.9 to 104.6 mg/kg/day for the Hsd rats and 104.6 to 104.6 mg/kg/day for the F344 rats. F344 rats had higher concentrations of deoxycholic acid than the other strains. In general, concentrations of cholic acid and chenodeoxycholic acid were similar among the strains.

**Strain** | **FBA** | **Cholic** | **Deoxy** | **Chen** | **Litho**
---|---|---|---|---|---
CD | 91.4 | 0.867 | 3.804 | 0 | 2.227
W1 | 67.9 | 0.404 | 2.797 | 0 | 2.518
LE | 97.1 | 2.087 | 4.293 | 0.354 | 3.917
HSD | 76.6 | 0.282 | 3.366 | 0.010 | 2.003
F344 | 104.6 | 0.506 | 6.753 | 0 | 3.059

A COMPARISON OF THE IN-LIFE PARAMETERS AND TUMOUR DATA FROM TEN GANG-HOUSED DIETARY TUMOURIGENICITY STUDIES USING THE SPRAGUE-DAWLEY WISTAR STRAIN IN INTERNATIONAL GENETIC STANDARD OR HAN WISTAR STRAINS OF RAT.

W. N. Hooks and S. M. Harley. Toxicology/Theology, Huntington Life Sciences, Huntingdon, United Kingdom. Sponsor: C. Attenwill.

The in-life and tumour data were reviewed from the control groups of ten gang-housed dietary tumorigenicity studies conducted over 1994 to 1999. Five studies using the Sprague-Dawley International Genetic Standard rat (SD IGS) from Charles River, UK were compared with five studies using the Han Wistar rat from BioResearch Laboratories, Switzerland. Low protein maintenance diet was used in these studies. The comparisons have shown that the percentage mortality at 2 years for the Han Wistar rat studies (18% in males and 26% in females) was considerably lower than that of the SD IGS rat studies (57% in males and 66% in females). The bodyweight and food consumption data obtained for the Han Wistar rats were also considerably lower in comparison with the SD IGS rat. The most prevalent tumours in both groups were lymph node haemangiosarcoma in Han Wistar male rats. In conclusion, the Han Wistar rat has a satisfactory tumour profile and, when compared with the SD IGS rat, a high survival rate. Additionally, on the basis of regulatory requirements to reach 2 years with an adequate number of survivors the Han Wistar rat would be a suitable strain to use without recourse to diet optimisation methods.

APPLICATION OF UNRESTRICTED WHOLE BODY BIAS FLOW PLETHYSMOGRAPHY TO MEASURE RESPIRATORY PARAMETERS IN THE CONSCIOUS RAT.


It is essential that potential undesirable effects on the respiratory system of new pharmaceutical, veterinary or agricultural products, are properly evaluated prior to exposure to humans. In this context, the revised ICH Guideline on Safety Pharmacology Studies for Human Pharmaceuticals (S7A) identify assessment of in vivo effects on the respiratory system, as part of the Safety Pharmacology core battery. To meet the S7A guidelines and as part of our safety pharmacology R&D programme, the Department of Pharmacology has developed procedures for the measurement of a number of respiratory parameters in conscious, freely moving rats. The equipment (Buxco Electronics) comprises whole body plethysmographs connected to a data acquisition computer enabling real time analysis (using Biosysnom XA software). This allows the determination of a number of respiratory parameters including respiration rate, tidal volume and minute volume. Preliminary baseline studies showed that there were no statistically significant differences in respiratory parameters between Sprague-Dawley and Wistar rats, even of the time of day of recording although not surprisingly, there was evidence of habituation with repeated and prolonged exposure. To demonstrate ability of the system to detect drug effects on respiratory parameters, studies were conducted using morphine sulphate and theophylline. administered as single dosages either by oral gavage or intravenous injection, to male and female Wistar rats. As expected, relative to vehicle-treated animals, morphine depressed respiration whereas theophylline increased respiration rate with consequent increases in minute volume. These drug effects confirm the ability of whole body plethysmography to detect potential side effects of substances on the respiratory system in freely moving rats. The methodology ideally meets the ICH guidelines for an in vivo core safety pharmacology study using conscious animals under the preferred conditions of non-restraint.

LOCAL TOLERANCE STUDIES: HOW TO REDUCE THE NUMBER OF ANIMALS?


For oral compounds (i.e. bioavailability study in man) local tolerance is usually assessed in rat. For iv compounds, general toxicology studies can be used to assess this. For compounds infused in non-clinical trials via central veins, local tolerance may have to be assessed in a peripheral vein: first choice again is rat. Due to too long study duration and small size of rodent veins, other species could be preferred. Generally no bolus injections are performed but slow injections, or short or continuous infusions. Centrally a vena cava is used for continuous infusion. The anterior one should be avoided for local tolerance, because it is difficult to be sure that the tip of catheter is in vein and not in heart. Different injection sites can be used superficially for rats the tail vein is most marginal ear vein is generally used. Other superficial injection sites could be used to check the risk of misdirection. Several concentrations may have to be tested and placebo and ideally saline control groups should be added. The total number of animals can thus be quite high. Especially if several veins have to be used, several necropsy occasions are scheduled and/or further groups have to be added, taking into considera-
tion first findings (with first tested concentrations). We propose different ways to reduce this number. 1) Several veins from the same animal (e.g., one central and four superficial veins on different days) could be used, allowing evaluation of local tolerance at different times after treatment. 2) Animals implanted and dosed in different weeks (however needed for continuously infused animals, e.g., one week per concentration), allowing easier and better selection and avoiding use of less concentrated N some investigations could be performed during pilot escalating dose studies (e.g., on different days before necropsy); visual evaluation and grading of the external aspect of the superficial injection sites, and echography or MRI of superficial as well as central vessels.

1295

A PRIMARY SKIN IrrITATION STUDY OF ELECTROTRANSPORT SYSTEMS ON HAIRLESS GUINEA PIGS AND RABBITS.


The objective of this study was to evaluate the potential degree of dermal inflammation produced by three different E-TRANSS® (placebo) systems containing cetylpyridinium chloride (CPC) (0.04% or 0.2%) in the cathode hydrogel and one of two adhesives to adhere the systems to the skin. Each system had an anode and cathode. All of the systems had the same anode hydrogel formulation. Systems were applied to the intact dorsal skin of 12 hairless guinea pigs (1 or 2 systems/guinea pig) and 6 New Zealand White (NZW) rabbits (3 systems/rabbit). The total wearing time was approximately 1.5 hours. The direct current density was approximately 7.14 μA/cm² (based on a hydrogel area of 1.4 cm² and a total current of approximately 100 microamps) at both the anode and cathode. Application sites were scored for erythema and eschar formation and edema (modified Draize method) 0.5, 24, and 48 hours after system removal, and Primary Irritation Indices were calculated. No treatment-related changes occurred in body weights or clinical conditions. No mortality occurred. The Primary Irritation Indices are presented below. Histopathology results supported the mild irritation observed. In conclusion, the cathode hydrogels containing 0.04% or 0.2% CPC were mild irritants in guinea pigs and rabbits. The anode hydrogel was a mild irritant in guinea pigs and a non-to mild irritant in rabbits.

<table>
<thead>
<tr>
<th>Cathode Formulation</th>
<th>Anode: Hydrogel</th>
<th>Cathode: Hydrogel</th>
<th>Anode: Hydrogel</th>
<th>Cathode: Hydrogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04% CPC/Adhesive 1</td>
<td>1.3</td>
<td>1.4</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>(mild; n=5)</td>
<td>(mild; n=5)</td>
<td>(mild; n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2% CPC/Adhesive 1</td>
<td>1.0</td>
<td>1.3</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>(mild; n=6)</td>
<td>(mild; n=6)</td>
<td>(mild; n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24% CPC/Adhesive 2</td>
<td>1.0</td>
<td>1.3</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>(mild; n=6)</td>
<td>(mild; n=6)</td>
<td>(mild; n=6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1296

ASSESSMENT OF RETINAL DEGENERATION IN OUTBRED ALBINO MICE.


Complete evaluation of a drug’s safety necessarily include an ophthalmic assessment. Various stocks of mice are typically used in nonclinical safety studies. However, despite the commonality that breeders attempt to maintain in outbred populations, certain genetic constitutions develop that provide sensitivities of which both breeders and toxicologists are unaware. Increased sensitivity to light in a laboratory animal can confound the interpretation of ophthalmic data generated from a particular study. Retinal degeneration (RD) was observed to occur in outbred albino mice (Hsd:ICR.CD1). Such a pathologic manifestation has been reported to be associated with an autonomic recessive genetic rd, retinal degeneration. In an effort to evaluate the genetic basis for the observation of RD in Hsd:ICR.CD1, a study was conducted comparing the light sensitivity of the following stocks: strains of mice: Hsd:ICR.CD1; Hsd:NIASCF-1; Hsd:NIASCF-1; Hsd:NIASCF-1; C57BL/6j-Tyr®r×2. In the response, consideration of the measurement under evaluation, ranges of natural variation and the overall biological plausibility of the observation. In discriminating between the reverse and the non-adverse effect it is important to consider: whether the effect is an adaptive response, whether it is present, the magnitude of the effect, its association with effects in other related endpoints, whether it is a precursor to a more significant effect, whether it has an effect on the overall function of the organism, whether it is a specific effect on an organ or organ system or secondary to general toxicity or whether the effect is a predictable consequence of the experimental model. In interpreting complex studies it is recognized that a weight of the evidence approach, combining the criteria outlined above to reach an overall judgement, is the optimal way of achieving the process. It is believed that the use of such a scheme will help to improve the consistency of study interpretation that is the foundation of hazard identification and risk assessment.

1297

BIOPHOTONIC IMAGING IN HO-LUC TRANSGENIC MICE: REAL-TIME DETERMINATION OF GENDER-SPECIFIC CHLOROFORM-INDUCED RENAL TOXICITY.


The metabolism of luciferin in mice transgenic for luciferase (HO-luc) produces light that may be detected in vivo by an intensified CCD camera (biophotonic). Thus the transgenic of luciferase-luciferase animals for genes regulated by specific toxic processes, coupled with real-time in vivo evaluation of specific gene expression may provide novel, non-invasive in vivo biomarkers which are predictive of developing toxicity. As part of a program to evaluate the potential of biophotonics for predictive toxicology we have conducted a series of studies in HO-luc mice, transgenic for the mouse hormone oxytocin-1 promoter (Xenogen, CA). Chloroform induced renal toxicity shows a marked gender difference in mice, with males being much more sensitive than females due to the expression of CYP2E1 in male mouse kidney. CYP2E1 mediated oxidative metabolites converts chloroform to phenol and results in covalent binding to proteins and depletion of glutathione. However, when female mice are pre-treated with testosterone the expression of renal CYP2E1 removes the gender difference and the kidneys become susceptible to chloroform toxicity. As part of the evaluation of HO-luc Tg mice, male and female animals were treated with chloroform (200 mg/kg, p.o., daily for 5 days) and imaged 2 and 6 hours after dosing. During a two-day washout period, female animals were treated daily with testosterone prior to repeat administration of chloroform for a further 5 days. Comparison of the in vivo response of the luciferase reporter with markers of toxicity measured ex vivo (differential gene expression of antioxidant system response genes, clinical chemistry and pathology) confirms the gender specific difference in chloroform renal toxicity in HO-luc Tg mice, and correlates with the expression of the endogenous HO-1 gene. These studies demonstrate the capacity of biophotonics for real-time site-specific gene expression, which may be predictive of developing toxicity.

1298

INTERPRETING TOXICITY STUDIES: RECOGNISING EFFECTS AND DIFFERENTIATING BETWEEN ADVERSE AND NON-ADVERSE EFFECTS.

R. W. Lewis, P. Billington, E. Debywegu, A. Gamers, B. Lang, I. van Millers and F. Carpanini. Health Assessment, Syngenta CTL, Macclesfield, United Kingdom, 1Agroecosystem, Dow Agroecosystem, Oxford, United Kingdom, 1Toxicology, Avetis Crop Protection, Sophia Antipolis, France, 1Toxicology, BASF AG, Ludwigshafen, Germany, 1HSA, Syngenta AG, Basel, Switzerland, 1TRS Services Inc, Charlotteville, VA and 1ECTO, Brussels, Belgium.

It is difficult to find criteria that can be used to guide consistent interpretation of toxicity studies. A two step process is outlined to address this issue. Differences from control values are initially evaluated in order to decide if they are treatment related or if they are chance deviations. In the second step, only those differences judged to be effects are further evaluated in order to discriminate between those that are adverse and those that are not. In differentiating an effect from a chance finding, criteria used to aid in this determination are: Dose-Response, consideration of the literature, the measurement under evaluation, ranges of natural variation and the overall biological plausibility of the observation. In discriminating between the adverse and the non-adverse effect it is important to consider: whether the effect is an adaptive response, whether it is present, the magnitude of the effect, its association with effects in other related endpoints, whether it is a precursor to a more significant effect, whether it has an effect on the overall function of the organism, whether it is a specific effect on an organ or organ system or secondary to general toxicity or whether the effect is a predictable consequence of the experimental model. In interpreting complex studies it is recognised that a weight of the evidence approach, combining the criteria outlined above to reach an overall judgement, is the optimal way of achieving the process. It is believed that the use of such a scheme will help to improve the consistency of study interpretation that is the foundation of hazard identification and risk assessment.

1299

APPLICATION OF IN SILICO APPROACHES TO TOXICITY PREDICTION.


In recent years there has been an increasing requirement for the early selection of lead drug candidates as a way to improve the efficiency of the drug discovery process. This has led to the emergence of high throughput screening assays for...
1300 SAFETY EVALUATION OF PHENOL AND M-CRESOL AS INACTIVE INGREDIENTS IN SUBCUTANEOUS DOSAGE FORMULATIONS.

G. H. Good fellow1, E. Cheng2 and J. M. Daniels1,2,1Pharmaceutical & Healthcare Group, Cantox Health Sciences International, Mississauga, ON, Canada and 2Pharmaceutical & Healthcare Group, Cantox Health Sciences International, Bridgeview, NJ

Phenol and m-cresol exhibit a wide range of overlapping commercial applications as demonstrated by their usage in the pharmaceutical, chemical, and agricultural industries. In pharmaceutical applications, their primary functional role is the antiseptic or preservative preservative properties of both compounds. Extensive use of these components in approved subcutaneous dosage forms is well documented in the US Food and Drug Administration's Inactive Ingredients Guide at levels up to 5% for phenol and 0.31% for m-cresol. In order to understand more fully any potential risks associated with the use of these compounds, the comparative safety of phenol and m-cresol was investigated. In acute subcutaneous toxicity studies performed in mice, rats, guinea pigs, and rabbits, the LD50 values for phenol and m-cresol were comparable and in excess of 300 mg/kg. Similar toxicological profiles (reduced water consumption, body weight loss, and nephropathy) were observed in 90-day rodent toxicity studies. In F344 rats treated with 0 to 780 mg/kg/day phenol in drinking water for 90 days, the lowest-observed-adverse-effect level (LOAEL), based on decreased water intake and body weight gain, was 780 mg/kg/day. A LOAEL also based on weight gain reduction, was observed at 150 mg/kg/day in Sprague-Dawley rats administered m-cresol (0 to 450 mg/kg/day) by oral gavage for 90 days. It is concluded that the use of either of these compounds in subcutaneous dosage formulations is strongly supported by the similarity of the results in available animal data, and therefore, should be based on other considerations.

1302 13-WEEK GAVAGE TOXICITY STUDY OF 5-(HYDROXYMETHYL)-2-FURFURAL (HMF) USING F344 RATS AND 86CF1 MICE.

M. Hejma'skref, J. D. Johnson, S. W. Graves, A. W. Singer, M. R. Ryan, R. Irwin3, R. Chhabra4 and M. Vallant1. Toxicology, Battelle, Columbus, OH and 3National Toxicology Program, NIEHS, Research Triangle Park, NC.

HMF, a furan compound formed during the thermal decomposition of hexose sugars and carbohydrates, is ubiquitous in the human diet and a contaminant of various foodstuffs. Several studies involving short-term toxicity testing of glucose and fructose solutions by heating or autoclaving F344/N rats and 86CF1 mice (10 animals/group) were given HMF by gavage at doses of 47 (mice only), 94, 188, 375, 750, or 1500 (rats only) mg/kg, 5 days/week for 13 weeks. Controls received the vehicle only (deionized water). Survival decreased for the 1500 mg/kg group male (9/10) and female (7/10) rats. Group mean body weight decreased 14 percent (relative to control) for the male 750 mg/kg group mice. Rat urine volume increased (1500 mg/kg) and urine creatinine decreased (≥275 mg/kg, m: ≥750 mg/kg, f). There were no HMF-related changes in clinical observations and clinical pathology. Mouse kidney weights decreased and microscopic findings were limited to clear cytoplasmic vacuoles in the proximal tubular epithelial cells (≥188 mg/kg, m). Gross and microscopic findings were similar to control for the rats. (Supported by Contract No. N01-ES-15320.)

1303 ACUTE AND CHRONIC TOXICITY OF BITUMEN-SALT MASS END-PRODUCTS FROM THE DESTRUCTION OF RUSSIAN SARIN, SOMAN AND VX.


In support of Cooperative Threat Reduction, the Russian Federation (RF) (GosNIOKhT) participated in joint studies with the U. S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) to investigate the toxicity of materials leaked from bituminous salt masses (BSM) containing by-products from a chemical demilitarization process developed by the RF. These studies determined hazard categories for the BSMs and evaluated the effects of long-term exposure to leachates from the BSMs in animals. Acute oral toxicity values (LD50) were determined in rodents for the components of BSM resulting from the destruction of Russian sarin (GB), soman (GD) and VX. Inhalation studies in rats evaluated the toxicity of volatile components resulting from heating (up to 130 °C) the BSMs, a process similar to that involved in the second phase of BSM production. Chronic toxicity was also investigated over six months in rats. Using a leaching process developed by GosNIOKhT, aqueous leachate solutions were prepared from bitumen alone, or GB-, GD- or VX-BSM. For each leachate, five groups of animals were dosed orally five days per week. Three groups were administered leachate at concentrations of 100%, 20% or 4%. Two other groups served as controls and were exposed to either leachate derived from bitumen alone, or ground water. Various physiological, biochemical and hematological indices as well as histopathology were evaluated. Results indicated no adverse effects in rats following inhalation of volatile components or chronic oral exposure to leachates from GB-, GD- or VX-BSM. Data from these experiments placed each BSM into a low hazardous toxicity category (Category IV), avoiding any BSM standards. The data is supported by toxicity studies conducted by USACHPPM.

1304 NINETY-DAY SUBCHRONIC ORAL TOXICITY OF AQUEOUS LEACHATES DERIVED FROM BITUMINOUS-SALT MASSES CONTAINING REACTION BY-PRODUCTS FROM THE NEUTRALIZATION OF U. S. OR RUSSIAN NERVE AGENTS.


In support of Cooperative Threat Reduction activities, the U. S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) participated in joint studies with the Russian Federation (RF) to evaluate the subchronic oral toxicity of materials leaked from the bituminous-salt mass (BSM) end-products of a chemical demilitarization technology developed by the RF. Subchronic oral toxicity studies were conducted by USACHPPM to address concerns that materials leaked from the BSMs could pose a potential health threat to receptors in the vicinity of future storage/disposal sites in Russia. Aqueous leachate materials derived from Russian bitumen alone, BSM containing demilitarized U. S. manufactured sarin (GB), or Russian manufactured GB, soman (GD) or VX were prepared using a toxicity characterization leaching procedure (TCLP) and administered by gavage to adult male and female rats for 90 days. Dosage levels of leachate were set at 0, 4, 20, and 100%
of the test material, and were administered at 10 ml/kg body weight. Data included measurements of body weight, food consumption, serum chemistry, hematology, urinalysis, organ weights, histopathology, and immunotoxicology parameters. The results indicated that aqueous leachable materials derived from US GB-BSM, GB-BSM, GB-BSM, BF, BF-BSM, and BF-BSM induced no significant adverse health effects when administered subchronically to rats for the given test periods. This data supports and enhances the findings of additional joint toxicology studies conducted in Russia.

1305  GENOTOXICITY AND 90-DAY/DEVELOPMENTAL TOXICOLOGY STUDIES ON AN EXPLOSIVE FORMULATION.
H. D. Dodd, S. Sharma and G. M. Hoffman. AFRL/HEST, ManTech Environmental, Dayton, OH; ManTech Environmental, Research Triangle Park, NC and Huntington Life Sciences, East Millstone, NJ.

Composition B Replacement 12 (PAX-21) is a new explosive formulation that is being considered for replacement of the current explosive. Composition B (Comp B). PAX-21 differs from Comp B because it contains new components, such as 2, 4-dinitrotoluene (DNT) and ammonium perchlorate (AP). Genotoxicity and 90-day/developmental toxicology studies were performed to evaluate potential toxic effects of PAX-21. PAX-21, Comp B, and DNA were mutagenic to bacteria (Ames assay). However, these substances were not mutagenic to Chinese Hamster Ovary cells (AS52X/IPTG assay) and did not induce micronuclei in the Swiss CD-1 mouse polychromatic erythrocyte system. Mortality resulted in rats exposed for 2 weeks (6 hr/d, 5 days/wk) to aerosol concentrations of DNA (the most volatile fraction of PAX-21) ≥ 545 mg/m³. Mild signs of toxicity and non-specific minimal metaplasia of the lung tissue were observed at 165 mg/m³. Rats were dosed (gastric intubation) with control, 15, 30, or 90 mg/kg-d PAX-21 for 90 days under-conestion for general toxicity, including histopathology, necrosis, and blood chemistries. At 30 and 90 mg/kg-d, maternal signs of toxicity and slight fetal body weight alterations at 30 mg/kg-d, and no observable adverse effects at 15 mg/kg-d (NOAEL). In a prenatal developmental toxicity study, rats were dosed (gastric intubation) with 0, 15, 30, or 90 mg/kg-d PAX-21 on gestation days 6-19. Mortality was observed at 60 mg/kg-d maternal signs of toxicity and slight fetal body weight alterations at 30 mg/kg-d, and a NOAEL at 15 mg/kg-d. Results from these studies were used to propose an occupational exposure level of 0.35 mg/m³ for PAX-21. (Supported by US Army Product Manager for Mortars in collaboration with US Air Force AFRL/HEST.)

1306  APPLICATION OF TOXICITY IN THE DEVELOPMENT OF ENVIRONMENTALLY FRIENDLY ELECTRONIC COUNTERMEASURES.
D. P. Aftens, C. L. Wilson, K. R. Stiff, and B. J. Spanger. Naval Health Research Center Toxicology Detachment, WPAFB, OH and Naval Research Laboratory, Washington, DC.

Millions of thin, aluminum-coated fiberglass dipoles (i.e. "chaff"), ranging from 0.8 - 7.5 cm in length, can be deployed by military aircraft and vehicles to confuse enemy radar. Chaff is scored onboard vehicles in small cartridges that consist of a piston, 0.5-1.5 million chaff fibers, a cartridge body, an endcap, and either a pyrotechnic charge or spring-loaded release mechanism that forces the piston through the cartridge body resulting in the: liberation of the endcap and chaff dipole. The accumulation of scattered energy in the piston and dipole in the environment from chaff launching missions in a concern of the Defense Department of Environmental and other government agencies. The US Navy has embarked on a program to develop degradable chaff cartridges, pissons, and endcaps by the 2003 fiscal year. Five biodegradable materials are being considered for use. Degradability and environmental toxicity tests are helping to select the most suitable construction materials. Relative degradability of the materials is being evaluated by measuring total organic carbon (TOC) released by the materials over time in deionized water. Environmental toxicity testing of the biodegradation products from the five materials is being conducted in 2 terrestrial plant species and 7 species of aquatic organisms. Biodegradation products of each of the five biodegradable materials under degradation did not inhibit germination or affect shoot growth of Brassica rapa or Lepidium sativum significantly. Biodegradation products from 3 of the biopolymers were found to be toxic to aquatic organisms with LC50s ranging from 0.5 - 14.7 mg/l TOC. Information gained from these studies will be used for making decisions on which (if any) of the polymers will be suitable for the construction of biodegradable chaff cartridges, pissons, and endcaps. Tensile strength, ease of cast, production waste stream, and overall cost of manufacture are factors that also need to be considered.

1307  QUANTITATIVE DETERMINATION OF PETROLEUM HYDROCARBONS IN INDOOR AIR: AN ON-SITE TOOL FOR RISK ASSESSMENT.

Many homes in the United States are heated with #2 fuel oil. In northern climates, fuel oil storage tanks are often kept in basements to avoid freezing during the winter months. During catastrophic flooding events, these tanks may rupture and release their contents. The spilled fuel oil then assimilates into the building materials (e.g. wood and concrete) and subsequently desorbs into the indoor air over time. Methods for on-site, quantitative determination of petroleum hydrocarbons in air were explored. Currently accepted methodologies (e.g. USEPA standard methods) require that air samples, regardless of sampling method, be transported to an off-site laboratory for analysis. During this transport, the possibility of analyte loss is introduced, and inaccuracies may arise in the obtained results. Accurate measurement of the concentration of airborne pollutants is essential for the determination of the health risk to individuals. On-site analysis not only reduce the risk of analysis loss, the results are also immediately available, and appropriate actions (e.g. removal of individuals present) may be taken with little or no delay. Portable Gas Chromatography (GC) equipped with an absorbent resin (Tenax GR) trap was used for direct air sampling and chromatographic separation of gasoline, diesel fuel, aviation gas and #2 fuel oil. Analyses were also carried out with a handheld Photonics Detector (PID), which were compared to the GC results. The GC method showed comparable response factors for all test fuels, whereas the PID showed an increased response factor for gasoline. Both methods were linear over the tested range of concentrations, with good to fair reproducibility. A study of aging #2 fuel oil over a period of two weeks showed a fairly smooth drop in response using GC methods, and somewhat erratic response with the PID.

1308  EVALUATION OF POTENTIAL HEALTH EFFECTS FROM INHALATION EXPOSURE TO MYCOTOXINS IN INDOOR OFFICE AND RESIDENTIAL ENVIRONMENTS.

There have been numerous claims of human toxicity from molds in indoor office and residential environments, we created a simple inhalation exposure model based upon concentrations of representative mycotoxins in mold spores. The model used: 1) the amount of mycotoxin per spore as reported in peer-reviewed literature, 2) the greatest concentration of spores which, in our experience, could be credible to be present in continuously occupied space, 3) 100% retention of inhaled particles, 4) and 24-hour/day exposure duration. The maximum modeled exposures of some mycotoxins potentially found in molds that may grow indoors are: fumonisin A, 5.4 x 10⁶ mg/kg; aatrin H, 1.6 x 10⁵ mg/kg; and ochratoxin A, 5.6 x 10⁶ mg/kg. These exposures are 10⁻¹⁰ th less than reported lowest adverse effects levels in appropriate in vivo systems. Analogous assumptions can be used to establish a maximum modeled exposure of some better-studied and more toxic mycotoxins (not reported in offices or residences) to serve as a point of comparison. If molds produced the following mycotoxins in offices or residential environments, modeled exposures for aflatoxin B₁, ochratoxin A, and T-2 toxin would be 1.2 x 10⁵ mg/kg, 1.0 x 10⁴ mg/kg, and 9.1 x 10⁴ mg/kg, respectively. For aflatoxin B₁ and ochratoxin A, the modeled exposures are at least two orders of magnitude below those considered safe by the USEPA for the most sensitive human population (children) by ingestion and WHO for the most sensitive species by ingestion, respectively. For T-2 toxin, the modeled exposure for 24 hours is approximately 10⁻¹⁰ th less than the estimated human acute oral LD₅₀. Ingestion data were used because quantitative inhalation data are not currently available. It appears that even when using extremely conservative estimates, it is nearly impossible to inhale sufficient mycotoxin in office and residential environments to produce meaningful toxic effects.

1309  EVALUATION OF THE SUBCHRONIC TOXICITY AND ONE GENERATION REPRODUCTIVE EFFECTS OF A FLUOROALKYLTHYL URETHANE POLYMER.
G. T. Makovec, D. A. Dekker, L. Nyklestam and L. C. Steggler. General Toxicology, DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, DE.

A fluoralkylthyl urethane polymer, used as a carpet pronestant, was evaluated for its oral subchronic toxicity and reproductive effects when administered to male and female rats by gavage at doses of 0, 50, 250, or 1000 mg/kg/day. No compound-related mortality occurred in the study. No adverse clinical signs of toxicity or neurobehavior changes were observed in male or female rats in any dose group. Hepatic cellular hypertrophy was observed after 90 days of dosing in male rats at all

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weights, gross necropsy findings, and respiratory tract histopathology did not differ substantially among all smoke-exposed groups. All smoke-exposed groups exhibited a similarly reduced incidence and severity of respiratory tract histopathologic findings after a 4-week recovery period following the smoke exposure period. It is concluded that the addition of DAP to experimental cigarette at levels resembling or exceeding those reportedly employed in contemporary cigarette manufacture does not significantly affect a variety of indices of smoke inhalation exposure or effect in this subchronic rat model.

1312 SAFETY ASSESSMENT OF HIGH FRUCTOSE CORN SYRUP (HFCs) AS AN INGREDIENT ADDED TO CIGARETTE TOBACCO.


Cigarette design has evolved over a century, with many new technologies introduced in the last several decades. Modifications to cigarette designs have historically involved the incorporation of new ingredients, filters, and tobacco processes. The majority of ingredients are used to enhance aroma and flavor. Others are used to facilitate tobacco processing, facilitate cigarette manufacturing, and preserve moisture levels in the finished cigarette. RJR has developed a tiered testing strategy to evaluate the potential for new ingredients, tobacco processes, and technological developments to increase or reduce the biological activity of cigarette smoke or cigarette smoke condensates (CSC). The core of RJR's product stewardship program is comparative testing, typically including chemical and biological components. This comparative approach characteristically relies on the comparison of data from a test cigarette, incorporating the change, as compared to data from an appropriate control. As part of the ongoing stewardship efforts, cigarettes produced with high fructose corn syrup (HFCs), as added tobacco casing material, were subjected to an extensive evaluation. Basic components of this evaluation are an analysis of selected mainstream smoke constituent yields, an Ames assay in Salmonella typhimurium strains (TA98 and TA100), a sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells and a neutral red cytotoxicity assay in CHO cells, a 30-week dermal tumor-promotion evaluation of cigarette smoke condensate in SENCAR mice, and a 90-day inhalation study of cigarette smoke in Sprague-Dawley rats. The results from these assays demonstrate that the addition of HFCs as an alternate tobacco casing material, up to an inclusion level of 3% dry weight in the final blend, did not produce a differential response in smoke chemistry or biological activity when compared to CSC or mainstream cigarette smoke from control cigarettes without added HFCs.

1313 CALAMUS: REASSESSMENT OF CARCINOGENICITY.

S. H. Humphreys, P. N. Dua, W. Franklin-Carroll and P. M. Bolger, Risk Assessment, USDA, Washington, DC.

The flavoring agents, Calamus root and its constituents, were considered carcinogens because of reported association of ingestion in feeding andavage studies and intestinal (mesenchymal) cancers in the 1960's. Recent introduction of Calamus in dietary supplements again raises questions of potential hazards of ingesting Calamus. Osborne-Mendel rats were dosed with oil of calamus or "beta" ascarone, an aldehydic component of the oil, and not the plant itself. Often neither the species of Calamus was recorded or the level of ascarone, which ranges from 0 to 96% of the oil, depending on the cultivar and season, with the oil constituting up to 10% of the plant. Extant internal FDA reports reveal flaws in experimentation and interpretation. Absence of complete protocols, small numbers of rats used in the experiments, reporting bias, disease, poor survival, inadequate sampling of tissues for microscopic examination, and inadequate tissue preservation preclude definitive interpretation. Cardiac and hepatic lesions with chronic passive hyperplasia, growth depression, and mortality were reported in 18-week studies and most of the dose-response relationship claimed in 2-year carcinogenicity studies were in rats dosed well above the 18-week LD50. Examination of archived slides revealed pleomorphic, mesenchymal tumors, some similar to fibrous histiocytomas, a spontaneously occurring tumor type in Osborne-Mendel rats. Bias in over reporting tumors in experimental animals may explain why a positive control in one set of experiments was negative in another set. Other studies of carcinogenesis in mice after intraperitoneal injection of "beta" ascarone in 1987 and in 1999 described palpable hepatomas; but no histologic confirmation was made. The only known effect in humans is protracted emesis after ingestion of a whole rhizome. The relevance of these studies to occasional human oral ingestion of "beta" ascarone in the parts per billion range used for flavoring is equivocal. Available evidence is inadequate to establish the carcinogenic potential of Calamus root and its constituents.
PEROXIDE DEGRADATION KINETICS DURING USE OF CREST WHITESTRIPS.


Cavity and systemic exposure to peroxide are important measures of tooth whitening safety. This clinical trial evaluated the hydrogen peroxide (HP) degradation kinetics of 6.0% HP Crest Whitestrips (CWS) on the device, teeth, and in saliva during use of this polyethylene strip-based tooth bleaching system. This was a single center, cross-over, 12 subject trial where only maxillary teeth were treated. Device, tooth, and saliva peroxide concentrations were determined at four time points: 5, 10, 30, and 60 minutes. The median peroxide concentrations on the strips at 0, 5, 10, 30, and 60 minutes wear time were 6.1, 3.8, 3.4, 2.6 and 1.4%. The median peroxide concentrations on teeth at 5, 10, 30, and 60 minutes of strip wearing time were 4.0, 3.1, 3.0 and 1.7%. The median peroxide concentrations in saliva were 0.017, 0.004, 0.006 and 0.002% at 5, 10, 30 and 60 minutes.

The concentration peroxide on teeth was not significantly different from device. The HP concentration in saliva was significantly different from both device and teeth (p<0.0001 using a single ranked test). A comparison was made to a previous study with 5.3% HP CWS, 10% Opalescence (10% carbamide peroxide tooth whitening tray system, -3.3% HP) and 20% Opalescence (20% carbamide peroxide, -6.6% HP). Dose-Response relationships were observed for the device and teeth data with the 5.3% and the 6.0% HP CWS, as well as with the 10% and 20% Opalescence products. Overall, median salivary HP concentrations were less than 0.02% for any product at any time point measured. This clinical research confirms the safety of CWS by demonstrating the minimal systemic exposure and rapid degradation of HP during use.

FROM EPIDEMIOLOGY TO THE GENE: MECHANISMS BY WHICH PARTICULATE MATTER INDUCES CARDIOVASCULAR EFFECTS.

R. B. Devlin and K. L. Dreist.
NHEERL, EPA, Research Triangle Park, NC.

Much attention is currently focused on understanding the pathophysiological basis for the observed linkage between ambient air PM exposure and acute mortality. Although there are indications that PM may directly or indirectly affect the cardiovascular system, the biological mechanisms for most of these associations are unknown. A significant effort in PM health effects research has turned towards examining the toxicological mechanisms that underlie PM systemic effects. Recent epidemiological, clinical, and toxicology studies suggest that PM can induce changes in the autonomic control of the heart as well as alter blood components involved in the acute phase response, clotting and coagulation. This symposium will draw on recent cutting edge approaches that investigate PM effects of the cardiovascular system and will integrate information from panel controlled human exposure, animal and in vitro studies. The first two presentations will describe how PM induces changes in heart rhythm and blood components involved in inflammation, the acute phase response, clotting, and coagulation. The next two presentations will describe changes in the cardiovascular system of animals exposed to PM, and will contrast these results with changes found in humans. The final presentation will explore in vivo and in vitro cardiac molecular effects produced by combustion PM.

CHANGES IN CARDIAC BIOMARKERS ASSOCIATED WITH AMBIENT PARTICLES.

A. Peters1, A. Ibdul-Mallii2, R. Ruckerl1,2, G. Wolke1,2, M. Piriz1, W. Koenig1,2, V. Marcer1, W. Zarebsa1 and E. Wichmann1,2.1Institute of Epidemiology, GSF, Neuherberg, Germany. 2Ludwig-Maximilians University, Munich, Germany.

University of Ulm, Ulm, Germany. Department of Medicine, University of Rochester School of Medicine, Rochester, NY. Sponsor: R. B. Devlin.

Ambient particles have been associated with exacerbation of cardiovascular disease. However, the biological mechanisms leading from the deposition of ambient particles to acute coronary disease are still to be elucidated. A panel of 58 patients with coronary artery disease has been studied in Erfurt, Germany during the winter of 2000/2001. The goal of the study is to quantify the role of fine and ultrafine particles in elicitation of an acute phase response and changes of the autonomic nervous system function. Repeated clinical examinations were conducted including EKG recording, blood pressure measurements and blood sampling. Ambient particles were characterised by a laser spectrometer and a differential mobility analyser proving particle number concentrations in the size range between 10 nm and 2.5 μm. Mutivariate regression models using generalised linear models will be used to quantify the associations between physiological markers such as CRP protein or heart rate variability and ambient particle concentrations in different size ranges adjusting for time trend, meteological variables and viral infections. 863 examinations have been conducted over a half year period, which were more than 95% of the scheduled examinations. EKG readings and blood samples are currently being analysed. The contribution of the study to elucidate the biological mechanisms responsible for the observed health effects of ambient particles within a potentially susceptible subgroup of the general population will be discussed.

CARDIOVASCULAR CHANGES IN YOUNG AND ELDERLY HUMANS EXPOSED TO CONCENTRATED AMBIENT AIR PARTICLES (CAPS).

R. B. Devlin1, W. E. Cusco2, H. Kehl1 and A. J. Ghi0. 1NHEERL, EPA, Research Triangle Park, NC and 2Department of Medicine, University of North Carolina, Chapel Hill, NC.

Epidemiology studies have shown an association between ambient air particles (PM) and mortality related to cardiovascular causes. In addition, recent panel studies have shown associations between PM and decreased heart rate variability in elderly people with cardiovascular disease. Panel studies have also shown associations between PM and changes in blood factors involved in viscosity and coagulation. The purpose of this study was to determine if exposure of young and elderly volunteers to moderate levels of PM under controlled conditions results in decreased heart rate variability and increased blood factors involved in coagulation. Twenty healthy young volunteers (aged 18-35) and 20 healthy elderly volunteers (aged 65-80) were exposed to concentrated Chapel Hill ambient air particles (CAPS) for two hours. Heart rate variability was determined before, immediately after, and 24 hours after exposure. Decreases were observed in both time and frequency domains immediately following CAPS exposure in the elderly, but not young, subjects. No changes were apparent in either group 24 hours following exposure to CAPS. Both young and elderly subjects had increased blood factors involved in coagulation 24 hours after CAPS exposure. These data confirm and extend findings reported in panel studies and suggest a possible mechanism by which PM may cause increased cardiovascular mortality. This abstract of a proposed presentation does not necessarily reflect EPA policy.

EFFECTS OF EXPOSURE TO PARTICULATE AIR POLLUTANTS IN ANIMALS WITH CARDIOVASCULAR DISEASE.

C. Nadziejko, T. Gordon and L. C. Chen. Environmental Medicine, NYU School of Medicine, Tuxedo, NY.

We have examined the effects of particulate air pollutants (PM) in normal rats and rats with cardiovascular disease to determine whether PM can cause adverse effects in cardiac function in the absence of exacerbation of respiratory disease. Normal young rats, old rats (18 months old), and spontaneously hypertensive rats (SHR) were implanted with either EKG transmitters or blood pressure transmitters. A single 4-6 hr exposure to concentrated ambient PM (CAP) caused a significant change in heart rate in young, old and SHR rats that was not associated with a change in body temperature. No significant change in heart rate was seen in rats exposed to 25 ppm Co., 1 ppm NO2 (SHR rats) or 1 ppm SO2 (young and old rats). Exposure of old and young rats to ultrafine carbon (generated by pyrolysis of acetylene) caused changes in heart rate that were similar to that seen with exposure to CAP. The time course and duration of heart rate change was independent of particulate exposure concentration or particle size (CAP or ultrafine carbon). In young rats, heart rate changes occurred within about 6 hours after exposure and lasted for about 24 hrs but in old rats the onset of heart rate changes lagged about 24 hrs after exposure and was shorter in duration. The old rats were found to have significantly more spontaneous arrhythmias than young rats. Exposure to CAP (but not ultrafine carbon or SO2) significantly increased the frequency of sinus arrhythmias in the old rats. These results suggest that adverse cardiac effects can occur following exposure to PM in rats with underlying cardiac disease without co-existing respiratory disease. Supported in part by EPA R827351.

CARDIOPULMONARY CHANGES IN PIGS EXPOSED TO RESIDUAL OIL FLY ASH (ROFA).

W. E. Cusco1, T. Johnson1, A. J. Ghi0, T. Huang1, D. L. Genter0, P.A. Bromberg2 and R. B. Devlin1. 1NHEERL, USEPA, Research Triangle Park, NC and 2Department of Medicine, University of North Carolina, Chapel Hill, NC.

Changes in cardiopulmonary function may contribute to mortality associated with ambient particulate matter (PM). In this study young pigs weighing 30 kg were mechanically ventilated and exposed to 2 mg/kg body weight nebulized ROFA suspended in saline, or to saline alone. Supplemental oxygen was used to maintain O2 saturation >90%. Changes in heart rate, aortic, central venous, and pulmonary artery pressures, cardiac activation and recovery intervals (ARIs) - an index of cardiac
and Sp4 exhibits tissue specificity for the brain. The interactions of Sp1 with cell cycle regulatory proteins, including p53, retnoblastoma protein, and cyclins is consistent with its developmental role. Furthermore, Sp1 has been shown to play a critical role during the differentiation of oligodendrocytes in the human brain. This presentation will discuss metal-induced changes in the activity and structure of this transcription factor family. Specific focus will be placed on the different roles of the various members of the Sp family in the regulation of gene expression.

1320 BIOAVAILABLE CONSTITUENTS MEDIATE CARDIAC MOLECULAR EFFECTS OF PULMONARY DEPOSITED FUEL OIL COMBUSTION PARTICLES.

K. Ducheck1, R. Jacko1, J. Richards1 and T. Knuckel1. USEPA, Research Triangle Park, NC, and North Carolina State University, Raleigh, NC.

Environmental health effects studies have shown the ability of particulate air pollution to affect cardiac autonomic control. The mechanisms by which particulate air pollution alters cardiac physiology are unknown. It is also not known what cardiac molecular effects, if any, may relate to specific combustion particles present within the fine fraction of particulate air pollution. This study examines the ability of fuel oil combustion particles, residual oil fly ash (ROFA), deposited in the lung to induce cardiac molecular effects and, if so, compared with pig exposure to these effects are assessed. Gene expression profiling detected an increase in mRNA levels for several cytokines and matrix metalloproteinases within hearts recovered from ROFA-instilled rats at 1- 6h and 24h post-exposure. Protein profiling detected an increase in the total phosphotyrosine protein content and the activation of several proteins targets from hearts recovered from ROFA-exposed rats as early as 0.25 - 1h post-exposure, indicating a rapid alteration in cardiac intracellular signaling homeostasis. Pulmonary exposure to ROFA did not increase plasma levels of IL-1beta, TNFalpha, IL-10 or endothelin-1. Elevated plasma vanadium (V) content was observed in ROFA-exposed rats as early as 15 min post-exposure which remained elevated up to 6h post-exposure. Decreasing trends control saline levels by 24h post-exposure. Exposure of cardiac fibroblasts/myocyte co-suspensions to a particle free ROFA leachate produced similar molecular responses as observed in vivo. These results demonstrated the ability of ROFA to produce cardiac molecular effects that were temporally correlated with plasma V content. These effects occurred rapidly and prior to the development of pulmonary inflammation, and could be replicated in vitro. These findings suggest that bioavailable metal constituents are responsible for the cardiac molecular effects following in vivo or in vitro exposure to fuel oil combustion particles.

1321 TRANSCRIPTIONAL INVOLVEMENT IN NEUROTOXICITY.

N. H. Zawia. Biomedical Sciences, University of Rhode Island, Kingston, RI.

It has become more apparent that exposure to various chemicals and environmental stressors elicits changes in the expression of a variety of genes. The study of gene expression and transcriptional regulation is an important aspect of understanding the mechanisms associated with neurotoxicity. The availability of whole genome sequences and the development of new tools to identify and monitor transcriptional activity have accelerated the rate of discovery. This has led to the identification of new genes and the ability to monitor the activity of multiple genes that are in concert in response to a stimulus. This symposium will deal with recent advances related to the elucidation of genes expression associated with the neurotoxic event as well as deciphering of signal transduction/transcription coupling that is altered following exposure to neurotoxic agents.

1322 THE SP FAMILY OF TRANSCRIPTION FACTORS MEDIATE METAL-INDUCED CHANGES OF GENE EXPRESSION.

N. H. Zawia. Biomedical Sciences, URI, Kingston, RI.

Sp1 is a transcription factor which contains a zinc finger motif and whose activity is modulated both in vivo and in vitro by heavy metals such as Pb, Hg, and Cd. Some of the genes under Sp1 control include ornithine decarboxylase, myelin basic protein, proenkephalin protein, NMDAR1 subunit, and metallothionein. It has now become apparent that Sp1 belongs to a family of proteins whose members include Sp2, Sp3, Sp4. All the Sp factors recognize the same DNA element. While the role of Sp2 is unknown, Sp3 is suspected of being a repressor modulator of transcription and Sp4 exhibits tissue specificity for the brain. The interactions of Sp1 with cell cycle regulatory proteins, including p53, retnoblastoma protein, and cyclins is consistent with its developmental role. Furthermore, Sp1 has been shown to play a critical role during the differentiation of oligodendrocytes in the human brain. This presentation will discuss metal-induced changes in the activity and structure of this transcription factor family. Specific focus will be placed on the different roles of the various members of the Sp family in the regulation of gene expression.

1323 GENE EXPRESSION AND CELL-SIGNALING EVENTS ASSOCIATED WITH TOXICANT-INDUCED GLIOSIS.

J. P. O'Callaghan1, K. Srinir1, M. L. Lasser1 and D. M. Felschow1. Molecular Neurotoxicology, CDC-NIOSH, Morgantown, WV and Ciphergen Biosystems, Fremont, CA.

Astrogliosis represents a sensitive and early response to the nervous system to all types of neurotoxic injuries. The generality of the glial reaction, despite the target selectivity of specific neurotoxic insults, implies that there are common "signals" underlying this cellular response. The discovery and characterization of such signals would, therefore, broaden our understanding of molecular mechanisms underlying diverse neurotoxic responses and lead to early "predictors" of neurotoxic outcomes. We present evidence for candidate genes and signaling pathways underlying the glial response to neuronal damage resulting from exposure to the dopaminergic neurotoxicant, MPTP. Our model, a single 12.5 mg/kg dose of MPTP to the C57 Bl6j mouse results in a 50% decline in dopamine and TH protein. The accompanying astrogliosis, as assessed by immunohistochemical GFAP, begins at 12 h and peaks at 48 h post-dosing. Striatal homogenates prepared from mice sacrificed by focused microwave irradiation to preserve steady-state phosphorylation revealed activation of the JAK/STAT pathway in the earliest phase of MPTP-induced gliosis. This effect was not observed in non-target regions and was completely reversed by neuroprotection with Nimexine. These results were indicative of effects specific to the neurotoxic condition and implicated potential upstream effectors in the JAK/STAT kinase modules, such as cytokines and trophic factors acting through the gp130/Ras pathways. Gene array analysis revealed enhanced expression of TNF-alpha mRNA and the Cyphepen Protein Chip2Platform of SELDI-TOF/MS analysis revealed a striatal-specific induction of 18 kD proteins consistent with induction of TNF-alpha. TNF-α receptor deficient mice showed complete neuroprotection against the neurotoxic effects of MPTP. Together these data show that a gene–protein– phospho–specific antibody "arrays" can reveal novel mechanisms potentially underlying the glial response to neurotoxic insult.

1324 GENE EXPRESSION PATTERNS IN LEAD NEUROTOXICITY.

T. B. Gullatte. Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

Human studies and animal models have clearly demonstrated the impact of developmental lead (Pb) exposure on behavior and cognitive function. Experimental animals exposed to environmentally relevant levels of Pb express deficits in tests of spatial learning and in synaptic plasticity such as long-term potentiation (LTP). The hippocampus is a brain structure essential for both of these processes and is known to be affected by Pb exposure. We have been interested in understanding the molecular basis associated with these deficits and have identified changes in the expression of specific subunits of the NMDA receptor in the hippocampus of Pb exposed rats. This presentation will discuss Pb-induced changes in the expression of selective genes associated with NMDA receptor-dependent signal transduction and calcium homeostasis. [Supported by NIH grant ES06189]

1325 INDUCTION OF TRANSCRIPTION FACTORS IN NEURONS THAT SURVIVE NEUROTOXICITY.

K. R. Pennypacker. Pharmacology USEF, Tampa, FL.

We report that levels of fos-related antigen-2 (FRA-2) are elevated long-term in several models of chemical-induced brain injury as well as after ischemia suggesting that this protein is involved in enhancing the transcription of genes related to the process of regeneration and repair. Tissueplasminogen, which regulates the degradation of proteins in the hippocampus and other limbic regions, results in a 5-fold induction of FRA-2 immunoreactivity in neurons in the pyramidal and dentate layers of the hippocampus starting at seven days post treatment and persisting for 60 days. Ischemic insult caused by middle cerebral artery occlusion results in an increase in FRA-2 expression the hippocampus ipsilateral to the ischemic lesion at 6 hours.

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after reperfusion. By one day, the increased expression is found in the neuronal layers of both hippocampi and its elevated expression lasts for at least 21 days. Methamphetamine and methylendioxyamphetamine, agents which cause degeneration of dopaminergic nerve terminals in the substantia nigra of the mouse, cause an increase in FRA-2 immunoreactivity which begins at three days post-treatment and returns to basal levels by day 5 and 15, respectively. Treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine caused elevated levels of FRA-2 in the mouse striatum at three days post-treatment. This abbreviated timecourse of FRA-2 induction in terminal degeneration is consistent with the evidence that this protein will increase to trimethylen and ischemia (cell death) but induction occurs during the period of regeneration and repair in both models. Thus, FRA-2 is induced in models of both cell death and terminal degeneration suggesting this transcription factor may serve as a universal signal for transcription molecules involved in the regulation of genes related to regeneration and repair in the mammalian nervous system. Work supported by AHA Nat. 9903072N and NIH NS39141-01A2.

1326 NUCLEAR FACTOR KAPPAB ACTIVATION PROMOTES SURVIVAL OF MOUSE HIPPOCAMPAL CELLS AFTER ACUTE TRIMETHYLIEN EXPOSURE.

C. A. Kissed and K. R. Pennycuick. Pharmacology and Therapeutics, University of South Florida, Tampa, FL.

Members of the nuclear factor-kappaB (NF-B) family of transcription factors play essential roles in diverse physiological processes including cell growth, differentiation, and development. A variety of external stimuli including pathogens, stress, and pharmacological agents can activate NF-B. However, the role of this transcription factor as a neurodegenerative or survival-promoting response to brain injury remains an area of discussion. To address this controversy, we have established transgenic mouse models of neurodegeneration using trimethylen, the well-characterized neurotoxicant with exquisite limbic selectivity. Neurodegeneration is exacerbated in the hippocampi of mice lacking the p50 subunit of NF-B. Activation of this transcription factor in response to an i.c.v. systemic injection of TMT is shown in mice carrying a B-dependent laBZ (galactosidase) reporter gene. Granule cells of the murine dentate gyrus subregion are particularly vulnerable to TMT toxicity, but neurons expressing activated NF-B, identified by galactosidase reporter activity, survived the injury seven days after treatment. Additionally, Fluoro-Jade, the histochemical marker of degenerating neurons, does not label cells immunoreactive for γ-galactosidase. These results further support a role for NF-B transcription factor in neuronal survival. This work was supported by grants from American Heart Association National 9930072N and National Institutes of Health NS39141-01A2.

1327 APPLICATION OF GENOMICS FOR MECHANISM BASED RISK ASSESSMENT.

R. D. Tyler and D. F. Robinson. ILSI Health & Environmental Sciences Institute, Washington, DC.

Recent advances in genomic and proteomic research, coupled with the availability of novel tools and methods with which to analyze the products of altered gene expression, have provided new insights into mechanisms of toxicity evoked by xenobiotics. While these advances promise to revolutionize our ability to characterize hazard, one of the short-term needs is to establish a body of publicly available knowledge to serve as a foundation for applying the data generated by these new methods to risk assessment. The ILSI Health and Environmental Sciences Institute is providing a scientific forum to facilitate further development and advances in genomics and proteomics methodologies toward the goal of increasing the utility of gene expression data for mechanistic-based hazard identification and prioritization. One of the short-term goals of this endeavor is to develop a body of publicly available knowledge to serve as a foundation for assisting in the generation, interpretation and application of data generated by these new methods. To this end, a collaborative research effort has been undertaken to help establish appropriate experimental methodologies for measuring alterations in gene expression and to relate genomic and proteomic expression data to toxic endpoints characterized by established methods under standardized conditions. Through this collaboration, data are being generated in government, industry and research laboratories on a series of prototype compounds across a broad range of available microarray platforms. This workshop will present the outcome of the initial phase of this project which has focused on neurotoxicity, hepatotoxicity, and genotoxicity as important endpoints for the risk assessment of many chemicals. Presenters will discuss the issues associated with drawing correlations between patterns of gene expression with key biological parameters for these endpoints, insights gained into the mechanism of action of the compounds studied, the influence of specific experimental conditions on data variability and reproducibility, and other issues associated with interpretation and future application of data from these types of studies for risk assessment.

1328 FUTURE DIRECTIONS FOR SCIENTIFIC VALIDATION AND APPLICATION OF GENOMICS APPROACHES IN RISK ASSESSMENT.


The ILSI Health and Environmental Sciences Institute is providing a scientific forum to facilitate further development and advances in genomics and proteomics methodologies toward the goal of increasing the utility of gene expression data for mechanistic-based hazard identification and prioritization. These methodologies have the potential of the short-term goal of this endeavor is to develop a body of publicly available knowledge to serve as a foundation for assisting in the generation, interpretation and application of data generated by these new methods. To this end, a collaborative research effort has been undertaken to help establish appropriate methodologies for measuring alterations in gene expression by developing and applying standard protocols across multiple technical platforms. The initial phase of this project has focused on neurotoxicity, hepatotoxicity, and genotoxicity as important endpoints for the risk assessment of many chemicals. The successful completion of these initial studies has raised important technical issues relating to microarray applications including experimental design, sources of variability, data normalization, statistical methods and the sharing and comparison of results from different technical platforms. To facilitate the sharing of multi-platform results, the ILSI subcommittee has developed short-term microarray data exchange and storage formats, which allow simplistic comparisons between multiple datasets. It is clear that the most likely value from these approaches will be obtained when they are applied in concert with more "traditional" measurements of toxicant action such as well-characterized biochemical or histopathological markers or endpoints. In addition, the successful interpretation of changes in global patterns of gene expression will be greatly facilitated by the availability of an appropriately curated and carefully annotated public domain database of gene expression data with simultaneous recording of appropriate phenotypic markers related to the effects of chemical exposure. Ongoing effort and challenges in these areas will be presented.

1329 GENOMIC AND PROTEOMIC ANALYSIS OF ACUTE AND SUBCHRONIC NEPHROTOXICITY.

T. A. Bertram. Drug Safety, Pfizer, Groton, CT.

Pharmacologist accounts for about 40% of clinically relevant acute renal failure/nephrotoxicity accounts for up to 60% of regulatory actions related to target organ toxicity. Nephrotoxicity is associated with 6 basic mechanisms. These mechanisms are active on the Glomerus, Tubules/collecting ducts &/or Interstitium. The Nephrotoxicity group of ILSI's Genomics Subcommittee tested dose/time-response relationships of 3 agents in SD rats: Gentamicin, Captopril & Parnycin. 4 genomics platforms were used to evaluate mRNA expression. Common sources of variation in genomic analysis included: inter-laboratory in-life studies, inter-laboratory mRNA arrays analysis, inter-animals &/or intra-arrays effects. Genes were found to be regulated in treated animals at all times. Microarray analysis demonstrated temporal/dose-response for genes functioning in cellular injury, molecular repair, inter-cellular signaling, cellular proliferation, apoptosis, inflammation, fibrosis, electrolyte transport & acute phase response. Classical gene markers for glomerular tubular damage (gammal gamma transpeptidase), collecting duct toxicity (Na-H exchange) & interstitial damage (KIM-1) were identified using microarray technology. Patterns of gene responses for known toxic mechanisms & functional categories were consistent with what is known about the mechanism of nephrotoxicity. Potential applications of genomic & proteomic studies of nephrotoxicity include early candidate selection & prioritization, animal model development, elucidation of mechanisms for tissue injury & response, and interspecies extrapolations.

1330 GENE EXPRESSION MONITORING USING MICROARRAYS AS AN APPROACH FOR ASSESSING HEPATIC TOXICITY.


Hepatic toxicity is the most common adverse effect leading to label warnings, use restrictions and market withdrawals for pharmaceuticals. Traditional experimental approaches can reveal dose-dependent toxicity in animal models but rarely indicate toxic mechanism and are equivocal predictors for human responses. Microarrays and related tools have shown promise for the rapid identification of toxic mechanisms and may have utility in more specifically predicting human responses. To be useful for risk assessment these tools must be challenged for reliability and inter-laboratory reproducibility of results. Towards this, the hepatotoxicity working group of the ILSI Genomics Subcommittee is evaluating responses in rats to two hepatotoxins using a standard experimental protocol. Cisplatin (25 and 250 mg/kg/d) and methyrapine (10 and 160 mg/kg/d) were administered by oral gavage for 1, 3 and
7 days. Studies for both compounds were conducted in two different laboratories, and traditional toxicity parameters were consistent with reported effects. RNA samples from liver were distributed for examination in 16 different laboratories using six different platforms and at least three different data analytical approaches. Gene expression results for both compounds show sufficient agreement between laboratories and across platforms to allow progression toward new approaches to toxicity evaluation. In most cases expression of known marker genes for these compounds was observed. Discrepancies for specific genes are largely due to differences in platforms (gene representation, annotation and specific sequences) and approach to data analysis rather than biological or inter-laboratory variability. This consortium effort provides an unprecedented opportunity to evaluate and compare biological, genomic and toxicological parameters across different laboratories and different analytical platforms. Though improvements are needed in annotation and in data analysis tools, it is clear that monitoring gene expression changes has value for identifying and assessing hepatic toxicity.

1331 ASSESSMENT OF THE UTILITY OF DNA MICROARRAYS AS A PRACTICAL TOOL FOR CHARACTERIZING GENOTOXICITY.


Genetic damage elicits a variety of stress-related signals that alter expression of genes associated with numerous biological pathways. It has been proposed that patterns of gene expression changes may be characteristic of specific classes of genotoxic compounds and can be used to classify agents with different mechanisms of action. DNA microarray platforms provide a promising technology to assess these changes. To test the hypothesis that gene expression patterns can be correlated with mechanisms of action of genotoxicants, the ILSI Genomics Subcommittee has investigated the gene expression profiles of several direct-acting genotoxins in two different cell lines (mouse lymphoma and human TK-6 cells). This multi-laboratory collaboration has afforded the opportunity to assess several factors of inter-laboratory variability associated with various aspects of the technology. Two time points were examined: 1) cells were treated for 4 hr and immediately harvested for RNA isolation, or 2) cells were treated for 4 hr and allowed to recover for 20 hr (24 hr time point) before being harvested. At least two concentration levels were chosen with one inducing 70-80% cytotoxicity with a robust induction of genotoxicity (determined by either a TK-6 gene mutation assay or a micronucleus assay) or one inducing limited cytotoxicity with a 2-4 fold genotoxic response. Overall, the data indicates that modest changes in gene expression (-3-fold) were common in the in vitro studies, and levels of test compound that produce low cytotoxicity appear to induce relatively few gene changes. Interpretation of the data is influenced by the filter criteria used to define a response. At present, it is uncertain that these modest changes represent key biological responses to damage in these cell lines or if other mechanisms apply. To fully utilize information from these studies, it will be critical to analyze data in a manner that identifies critical genes in biological pathways involved in a genotoxic response and to distinguish small, but real changes from background noise.

1332 USING MICROARRAY TECHNOLOGY IN SAFETY EVALUATION: WHERE DO WE GO FROM HERE?

J. L. Stewart, D. E. Watson and T. P. Ryan, Toxicology and Drug Disposition, Lilly Research Laboratories, Indianapolis, IN.

Expression profiling by microarray analysis significantly contributes to many areas of Toxicology including biomarker development and mechanistic studies. However, the promise represented by microarray analysis must be balanced against the significant challenges that exist at all levels of the technology. For example, the quality of the data must be consistent from chip to chip and Lab to Lab. In addition, for public data to be widely disseminated, hardware and software systems must manage and deliver complex data sets from many sources and platforms to multiple users via internet links. Additional complexity occurs in interpreting results. One must know the function of a gene to interpret the biological significance of a change in expression. Thus, functional annotation of genes is a significant hurdle for the field since correct assignment increases the likelihood that changes in gene expression will yield useful biological information. Unfortunately, many of the transcriptome are not annotated nor has functional been assigned to many novel genes. In addition, nomenclature often does not reflect function. These problems represent significant challenges if we are to develop databases into which many users can deposit data and from which useful biological information can be extracted. Nonetheless, reference sets of expression profiles developed around defined SARs do offer insights into mechanism, suggesting that databases of gene expression information have applications in risk assessment. The ILSI effort on microarray technology highlights the promise and problems inherent to microarray technology. Continued effort will contribute to international databases and provide a benchmark for standardizing microarray technology within the industry.

1333 APPRAOChES TO THE ASSESSMENT OF FOOD ALLERGICENICITY.

G. S. Ludija and W. Dang, 'DuPont Co., Haskell Laboratory, Newark, DE and USDAF, Washington, DC.

Food allergy is an important health issue with the prevalence of IgE antibody-mediated food allergies among adults being approximately 2% and nearly 5% in children. There is a need to assess the safety of foods deriving from genetically modified (GM) crops, including the allergenic potential of novel gene products. In January 2001, a Joint FAO/WHO Consultation developed a revised decision tree for the evaluation of the allergenic potential of GM foods. In addition to indirect measurements of allergic activity (amine acid sequence homology and structural similarity to known human allergens, together with resistance to pepsi digestion), the new hierarchical approach now recommends confirmation using serological screening (i.e., using targeted evaluation of the reactivity of novel proteins with IgE antibody from sensitized subjects) and relevant immunological assays. Presently, there are no in vivo or animal models that have been validated for the identification or characterization of potential protein allergens. This Workshop will gather researchers working on various strategies for assessing protein allergenicity to describe the current state of knowledge and progress that has been made in the development and evaluation of appropriate testing strategies and to identify critical issues that must now be addressed. For in vivo models of digestibility, bioinformatics, and risk assessment in the context of food allergy prevention and management clinically will be discussed. Both rodent and non-rorden models for the measurement of allergenic potential of GM foods will also be reviewed.

1334 ALLERGY ASSESSMENT OF GENETICALLY MODIFIED (GM) FOODS: WHAT ARE THE ISSUES?

M. P. Holappel, TERC, Dow Chemical Company, Midland, MI.

Prior to marketing, GM products are required to be evaluated for their safety, including the potential for allergenicity. The process by which allergy assessment is conducted has evolved under the guidance of a variety of expert scientific bodies, and the key steps in the current decision tree include the following: Is the source of the gene allergenic? (addresses the history of exposure and safety of the source of the gene). Is the amino acid sequence similar to any known allergen? (addresses the potential for broad homology of the protein to known allergens; search for 8 or more contiguous amino acids). Is the protein stable to pepsi digestion? (addresses the potential for absorption through the stomach). To date, approximately 70 GM products have been assessed for safety and approved for use in at least one country by regulatory authorities. While the current decision tree has been successfully applied, there are scientific issues requiring additional clarification including the following: the need to standardize the amino acid sequence analysis; the uncertainty about whether IgE epitopes are missed by the current search criteria; the need to standardize the pepsi digestion assay; the need for scientific consensus on what to do with stable proteins, the need for scientific consensus regarding the usefulness of broad serum screens; the need for more research to validate predictive animal models. The FAO/WHO 2001 report addressed many of these issues and made recommendations that could provide additional reassurance of allergenicity assessment. However, some of these recommendations cannot currently be integrated because of the unavailability of validated methods. Any change in the current methodology should include a development plan with clearly defined goals and criteria. The FAO/WHO 2001 decision tree does not allow for a GM food to be deemed of allergenic risk - a more realistic scenario is a low probability of allergenicity, which introduces a degree of uncertainty. Any safety assessment approach should enable its practitioners to make clear, consistent decisions based on sound scientific evaluations.
legens have been utilized since the introduction of the first biotech crops in the early 1990s. Recent progress in the understanding of both the natural history of food allergy and the characteristics which define food and other allergens has led to new insights into the potential predictive value of in vitro tools with a view towards preventing the development of new food allergies. The most advanced of these tools include 1) the adaptation of bioinformatics techniques; and 2) the refinement of two factors which influence protein exposure via foods to stability to digestion and protein levels. The positive and negative predictive values of these factors alone and in combination will be discussed. In addition, emerging trends in the characterization of food and other allergens, especially allergens responsible for Oral Allergy Syndrome (OAS), will be reviewed in a view to enhancing the risk assessment approach for foods derived from biotech crops.

1336 ANIMAL MODELS FOR THE IDENTIFICATION OF PROTEIN ALLERGENIC POTENTIAL: THE BALB/c MOUSE. J. Kimber and R. J. Dearman. Research, Syngenta Central Toxicology Laboratory, Cheshire, United Kingdom.

Food allergy is an important and common health issue, and there is consequently a need to identify and characterize the sensitizing potential of novel food proteins. One strategy we have adopted is to measure allergenic activity as a function of the ability of proteins to provoke antibody (IgG and IgE) responses in BALB/c mice; a criterion that is known to favour adaptive immune responses of the quality required for allergic sensitization. Emphasis has focused to date on an examination of humoral immune responses induced following systemic exposure of mice (by intraperitoneal [i.p] administration) to the test material. Under these conditions it has been found that proteins known to be associated with food allergy in humans (including for instance peanut proteins and ovalbumin) elicit both specific IgG and IgE antibody responses, measured using enzyme-linked immunosorbent and passive cutaneous anaphylaxis assays, respectively. In contrast, other proteins derived from foods believed to exhibit little or no allergenic potential (such as for instance an extract of potato proteins), although immunogenic in BALB/c mice (measured as a function of IgG antibody production), did not induce IgE responses at all, or were associated with only low grade IgE production. In comparative analyses, exposure of BALB/c mice to proteins by gavage was found to be less sensitive and discriminatory. Experience to date indicates that the inherent sensitizing potential of novel food proteins can be evaluated (identified and ranked) on the basis of antibody responses stimulated by parenteral exposure of BALB/c strain mice.

1337 DETERMINATION OF PROTEIN ALLERGICITY: STUDIES IN A BROWN NORWAY RAT FOOD ALLERGY MODEL. L. M. Knippels1, J. D. Aswood2, A. H. Penninks1 and R. E. Goodman2.

1) Experimental Immunology, TNO Nutrition and Food Research, Zeist, Netherlands and 2) Monzano Company, St. Louis, MO. Sponsor: W. Dong.

There is a need to identify and characterize the potential allergenic activity of novel proteins introduced into genetically engineered crops. Although structural and physico-chemical approaches have been described, including comparison of sequence and structure to known allergens, determination of the stability to digestion with pepsin, stability with heat, and the presence of glycosyls or disulfide bonds; none of these directly measure the ability of proteins to cause allergic sensitization. For that reason there has been a growing interest in the development of suitable predictive animal models. Although several animal models are under development, none of these models have been widely tested with a variety of allergenic and non-allergenic proteins. In our rat food allergy model, we expose young Brown Norwegian (BN) rats (high IgE responder strain) to food proteins by daily gavage dosing for 42 days without the use of adjuvants. The animals develop specific IgE responses against known food allergens. After an oral challenge in previously sensitized animals, increased gut permeability, drop in blood pressure, drop in breathing frequency, and increased release of Ret Mac Cell Protease II in vivo was observed. In recent studies with the BN rat food allergy model the animals were sensitized to common strong and non-allergenic protein to study the potential allergenicity of food proteins. Sensitizations were attempted orally both with and without the use of the oral adjuvant Cholera Toxin (CT) and parenterally with the use of alum as an adjuvant. The results indicate that at day 42 the immune responses in the BN rat can distinguish between strong and non-allergenic responses. Although not of the proteins induced 100% of the animals to respond after oral exposure there was a difference in response observed between the proteins as measured by either antigen and isotype specific ELISA and by PCA.

1338 NON-RODENT ANIMAL MODELS FOR ASSESSING PROTEIN ALLERGENICITY. R. M. Helm. Pediatrics Allergy/Immunology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, AR. Sponsor: G. Ladish.

In veterinary medicine, skin and gastrointestinal reactions to foods are commonly diagnosed in domestic animals such as pigs, calves, and dogs. An inbred Sprieland/Baenji colony with a genetic predisposition, significant history and cutaneous signs consistent with food-induced allergic dermatitis has been selected to study food-induced IgE-mediated allergy. Newborn pups, subcutaneously sensitized with allergens in alum and vaccinated with attenuated distemper-hepatitis vaccine respond with allergen-specific IgE, skin test reactivity, and histologic features that mimic human food allergy. A significant contribution of this model is that it mimics a proposed mechanism in children representing viral infections and a protein "bystander" antigen response. Young pigs experience allergic reactions to become tolerant and develop a naturally occurring food allergy model for studying food sensitization and gut-immune mediated disorders resembling human food allergic diseases. The pig mimics the human digestive and gut immunologic functions, including nutritional requirements, a transient porosity of the immature gut to dietary proteins, the distribution and maturation of intestinal enzymes, and the enteral absorption of antibodies. Neonatal piglets sensitized by either intraperitoneal or intranasal administration of peanut allergens in the presence of adjuvant followed by intragastric challenge with peanut meal provokes emesis, skin rashes, lethargy and respiratory distress. These two non-rodent animal models demonstrate skin test reactivity when challenged with the relevant allergen, show pronounced mucosal swelling and persistent erythema following endoscopic analysis of food challenge that is similar to that of human subjects undergoing diagnosis for food allergy. Continued efforts in these non-rodent animal models will provide valuable insight into the mechanisms of IgE-mediated allergic disease and be able to assist in the prediction of the relative allergenicity of novel proteins.


The comparison of two genomes provides insight into the function and organization of the shared genes. A genome wide comparison of available human (95%) and mouse (15%) sequence was performed to identify putative orthologous genes. Approximately, 95,000 conserved elements were identified that were greater than 100 bp and 62% of these regions contained an open reading frame. The mean identity of the conserved elements was 87.2 ± 4.2% which is consistent with previously described orthologous transcripts between both human and mouse. A subset of 2,000 putative orthologous elements were spotted on tandem microarrays (one human and one mouse) and used to evaluate conservation of expression patterns between organisms. Across a panel of 9 tissues, gene expression between mice and humans was largely conserved with no less than 6-fold difference between organisms and 74% expressed with less than 3-fold different. Of the genes expressed with greater than 10-fold difference, the majority were genes of unknown function (42%), followed by signal transduction (24%), metabolism (12%) and cytoskeletal (10%). Although this study extends the comparison between mouse and man to the genome to the transcriptome, it highlights the potential differences between the two species and provides an approach for evaluating cross-species extrapolation issues in toxicity at the molecular level. Preliminary experiments show similar transcriptional responses to phorbol esters between human HepG2 and mouse Hepa1 cells. Additional experiments are underway to compare human and mouse transcriptional responses to a variety of chemical treatments. We propose that such orthologous arrays will be useful for evaluating animal models for relevance to both human disease and toxicology.


The rat is extensively used in toxicological studies, partly, because the degree of evolutionary divergence is low enough to allow a cross-referencing to human and rat responses with respect to toxicological endpoints. Therefore, cross referencing molecular transcriptome data from studies with rat and human probes has many important applications. We have investigated the expression pattern of 1007 orthologous genes that code for detoxification, cell proliferation, tumor development,
In general, there was a good degree of concordance between the gene expression profiles generated at each site. Expected changes in gene involved in fatty acid metabolism (e.g., acyl CoA oxidase, hydroxyacyl CoA dehydrogenase, 3-ketocycl CoA thiolase, AIB), cell proliferation (e.g., topoisomerase II-alpha) and metabolism (CPY4A11) were observed. Real Time PCR was used to confirm modulations for selected genes. Although some discrepancies in microarray data between the two laboratories were observed, they appear largely due to different approaches to image and data analysis. In conclusion, toxicogenomics has the potential to provide new and refined approaches to hazard identification and safety evaluation based on the identification of biologically relevant markers of toxicity.

1343 GENE EXPRESSION RESPONSES OF PRIMARY HEPATOCYTES TO VARIOUS HEPATOTOXINS, R. Dunn1, A. Kalkuhl1, A. Field, M. Dorbel, T. Woodruff, R. Bader1 and T. Singer1. 1Phase-I Molecular Toxicology, Sandoz AG, NN and 2Pre-Clinical Drug Safety, Boehringer-Ingleheim Pharmaceuticals, Biberach, Germany.

Pharmaceutical companies are aggressively evaluating screening systems to shorten the time required to identify leads and simultaneously eliminate poor drug candidates. To this end, both in vitro cell culture systems and gene expression profiling are being widely used. Immortalized cell lines, while useful in certain situations, have not yielded the breadth of toxicological information required to aid decision making for lead optimization. Primary hepatocytes are currently being evaluated for use in toxicity screening. As a first step for determining the utility of expression profiling in primary hepatocytes, experiments with "paradigm" compounds that elicit well-understood toxicities in vivo. Primary hepatocytes were exposed to various hepatotoxins including allyl alcohol (AA), isoniazid (ISO), methotrexate (MTX), phenobarbital (PB), tacrine (TAC), trovan (TRO), and valproic acid (VPA). Cells were exposed to each compound for multiple dose and exposure times in order to put the gene expression responses into perspective. AA had only modest effects on gene expression possibly due to induction of aldehyde dehydrogenase that detoxifies acrolein, the toxic metabolite of AA. ISO did not have consistent effects on gene expression and alterations were modest. MTX induced genes indicative of an antioxidant response such as catalase and glutathione synthetase. PB was nearly without effect on these studies. TAC induced expression of glutathione synthetase in addition to genes involved in reducing oxidative stress. TRO induced several genes of unrelated function and the responses were highly dependent on exposure time. VPA induced several genes including those linked to peroxisome proliferation in a dose- and time-dependent manner. VPA treatment of rats in vivo is linked to peroxisome proliferation. Thus, hepatoksin gene expression profiles derived from treatment of primary rat hepatocytes can provide insight into toxic mechanisms.


In order to increase speed and depth of toxicological screening, we have established a toxicogenomics database, ToxExplain, that can be used to accurately predict and help classify potential toxicants using gene expression changes. Using the Affymetrix GeneChip® RGU_34 series microarray, we have collected expression information from over 1,000 rat liver samples for greater than 26,000 microarray elements per sample. Rats were treated with known hepatotoxins that exert pathologies such as necrosis, steatosis, cholestasis, and hepatitis along with appropriate controls. In general, samples from rats treated with vehicle, pharmacologic, or toxic doses of each compound were collected at three or more time points. Over 500 general marker elements and more than 1,000 compound/class-specific markers were found to be predictive based on discriminantitive abilities and determined to be predictive using randomization procedures. Predictive models were built using linear discriminant analysis (LDA). The results show essential predictions of toxicity using the markers determined to be discriminative, as well as for subsets of these markers. In addition, more specific classification of toxic compounds was achieved with compound- and class-specific markers. We are continuing efforts to validate our liver models, expand them into other organ systems and cell types, and investigate the mechanisms of action of marker genes.


In the toxicogenomics field, many debates have centered on whether injury to a specific area of the kidney can be detected by gene expression profiling of the entire organ. The feasibility of detecting toxicity in the kidney was evaluated by exposing...
Sprague-Dawley rats for 6 hrs, 24 hrs, and 7 days to multiple doses of either penicillinaminomucleotide (PAN), which induces gomulcerous injury, or cisplatin, which produces proximal tubular injury. Clinical chemistry and histopathologic examination indicated clear evidence of overt toxicity only after 7 days of exposure unlike the 6 and 24 hour time points. The study was stopped when no overt toxicity was noted. The kidneys from all time points and doses were analyzed by the Affymetrix RCGU. Subsets of genes that selected from statistically significant expression differences induced by PAN-treated rats from both the 6 and 24 hour were used to develop a predictive model, as we were interested in determining if acute expression changes can predict late effects. The PAN was selected as a positive control as it is known to cause histopathologic changes in the kidney. Gene expression alterations are present before overt toxicity is observed by classical parameters of toxicity. This suggests that expression profiling is a more sensitive measure of toxicity that is capable of predicting the manifestations of a toxic phenotype much earlier than classical techniques.

**1346**

**GENE EXPRESSION IN SPLEENS OF RATS DOSED WITH ESTRADIOL, HYDROXYUREA, OR TAMOXIFEN.**

G. M. Farris, Database, PHASE-1 Molecular Toxicology, Santa Fe, NM.

The differential gene expression in spleens of rats dosed with immunotoxins were evaluated to determine patterns related to tissue damage and mechanisms of toxicity. Rats received an intraperitoneal injection of estradiol, hydroxyurea or tamoxifen at two concentrations and then tissues were collected at 6, 24, and 72 hours later. Tissue was analyzed using the Affymetrix RCGU. The spleens were examined for specific areas of tissue damage. Gene expression alterations were present before overt toxicity was observed by classical parameters of toxicity. This suggests that expression profiling is a more sensitive measure of toxicity that is capable of predicting the manifestations of a toxic phenotype much earlier than classical techniques.

**1347**

**EVALUATION OF TUMOR-PROMOTING ACTIVITY BY GENE EXPRESSION PROFILING.**


The number of potential leads pharmaceutical companies obtain has increased steeply in the last years due to advances in combinatorial chemistry and high-throughput screening. We therefore developed our predictive toxicology platform that will help pharmaceutical companies to focus on as early as possible on the most promising candidates by excluding potentially harmful lead compounds. Carcinogenicity activity may be a knock-out criterion for a drug. While genotoxic activity can be detected in short-term assays (e.g Ames test), the detection of carcinogeticity by tumor promoting compounds represents a real bottleneck since long-term animal studies are necessary. Using our broad gene expression profiling technology platform we have analyzed liver samples of rats fed with a panel of tumor promoters of different mode of action classes (e.g. Phorbol, TPA, N-ethyl-N-nitrosourea) and compounds with cytotoxic activity (Panag, CCL). The two pairs of 2, 4- and 6-diamino- and dinitrophenols that have or do not have tumor promoting activity, respectively, were also included in our analysis. In phase 2, 22 genes were analyzed in our "grown" gene array. The analysis revealed that the selection of appropriate tumor-promoting compounds according to their mode of action. Thus, our study shows that gene expression changes induced by a certain chemical is characteristic of that chemical and that compounds with similar mode of action induce similar gene expression changes.

**1348**

**CROSS COMPOUND PREDICTIONS USING GENE EXPRESSION PROFILES FROM ACETAMINOPHEN OR CARBON TETRACHLORIDE, TWO STRUCTURALLY DISTINCT LIVER TOXICOANTS.**


It is essential to develop tools to rapidly determine if new chemical entities (CNEs) have the potential to elicit a toxic response in critical organs such as the liver or kidney. In order to address this need, Gene Logic has developed the ToxExpress™ reference database as a pre-clinical tool for ranking CNEs. Two classic liver toxins, acetaminophen (APAP) and carbon tetrachloride (CCL), were studied to determine if gene expression differences, measured by the Affymetrix RCGU. Subsets of genes selected from the gene expression alterations induced by the APAP-treated rats at 3, 6 and 24 hr time points. These genes were used to develop a predictive model. The model was tested against itself as well as CCL in order to determine the capacity of the model to predict samples as toxic or non-toxic. The APAP model was accurate in predicting itself and all the 3, 6, 24, and 48 hr CCL-treated samples correctly. Interestingly, the 1 hr and 72 hr CCL-exposed samples were predicted as non-toxic. These samples failed to show evidence of necrosis and steatosis, and 72 hr samples displayed features of fibrosis and regeneration. This study provides evidence that predictions between structurally distinct toxins are feasible.

**1349**

**DOSE-RESPONSE BEHAVIOR OF ANDROGENIC AND ANTIANDROGENIC CHEMICALS: IMPLICATIONS FOR LOW-DOSE EXTRAPOLATION AND CUMULATIVE TOXICITY.**


Concern has arisen regarding the shape of the Dose-Response curve in the low dose range for EDGs. Although it is generally assumed that no-cancer effects, including those induced by EDGs, display a threshold, several scientists have questioned the validity of this assumption. In regards to cumulative risk, it is generally assumed that dose-additivity prevails for chemicals that act via a common mechan-ism of action. A TEF (Toxic Equivalent Factor) approach for mixtures is valid if the dose-response curves for all the chemicals are parallel. This presentation will examine the dose-response behavior of several androgenic and antiandrogenic chemicals, in vitro, antiandrogenic chemicals like hydroxylumifluoride and M2, a vinclozolin metabolite, display antiandrogenic activity at low concentrations and AR agonist activity at high concentrations. U- or inverted U-shaped Dose Response curves have been described in similar AR agonists. In addition, many AR-mediated in vitro and in vivo effects do not display obvious thresholds. Furthermore, the dose-response curves for the antiandrogenic developmental effects produced by vinclozolin (V) administration reveal that the response to this AR antagonist do not display a common shape or TEFs. When V and progesterone (P, an AR agonist) are co-administered, the effects all appear dose-additive, suggesting that a single TEF could be used to assess cumulative toxicity. While cumulative developmental effects also were observed with coadministration of P and an inhibitor of fetal testosterone synthesis (de-nbutyl phthalate), it is evident that single TEF will not suffice because the relative potencies of these two chemicals varies from tissue to tissue. Our data indicate that cumulative adverse effects can be observed after coadministration of chemicals that affect the differentiation of the reproductive tract, regardless of the mechanism of action. Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect USEPA policy.

**1350**

**EVALUATION OF THE 15-DAY INTACT MALL ASSAY: CAPABILITY TO DETECT THE ANTI-ANDROGEN FLUTAMIDE.**

M. S. Marty, K. E. Stobbs and E. W. Carney, Toxicology & Environmental Research, The Dow Chemical Company, Midland, MI.

The Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) recommended that the 15-day intact male rat assay be evaluated as a possible alternate Tox screening assay to detect estrogen-competitive compounds (EACs). While many EACs have been tested in this assay, studies to address interlaboratory variation and route of exposure are warranted. To this end, male CD rats (n=15/group) were exposed to the anti-androgen flutamide (0, 0.5, 1, 10 or 50 mg/kg/day) by gavage for
15 days, then euthanized 2 hours after the final dose. Reproductive organ, accessory sex gland and thyroid weights were measured to detect endocrine effects. At 50 mg/kg/day, fluoxetine significantly reduced terminal body weight by 9% and increased relative liver weights by 17%. At 50 and 200 mg/kg/day, there was a dose-dependent increase in several organ weights, including liver and spleen size. Allometrically, there was increased organ weights in absolute and relative weights, 21 and 48% decreases in relative prostate weights, and 27 and 61% decreases in relative seminal vesicle weights. The body weight decreases exhibited by the high dose animals were not sufficient to account for these organ weight changes. The decreases in these organ weights were similar in magnitude to previous results with the intact male rats (O'Connor et al., 1998), with 20 mg/kg/day of fluoxetine given intraperitoneally decreased epididymal, prostate and seminal vesicle weights by 20.4 and 55%, respectively. Testes and thyroid weights were not altered in either study. These data show that the 15-day intact male assay can reproducibly detect the endocrine activity of fluoxetine across different laboratories and routes of exposure.

1351

HERShBERG ASSAY: DOSE RESPONSE AND COMPARISON OF TISSUE RESPONSES TO ANDROGENS GIVEN ORALLY AND SUBCUTANEOUSLY


Three experiments (Exp.) tested effects of androgen agonists testosterone propionate (TP) and methyl testosterone (MT) on male accessory sex tissues. The protocols were adapted from the OECD program for validation of the rodent HERShBERG assay: TP (250 mg/kg, 100 mg/kg, and 30 mg/kg/day and MT by gavage (p.o.) at doses of 0.1, 0.5, 1, 5, 10 and 25 mg/kg/day (10-6 group: Exp 2) were administered once daily for 10 days to 6-week-old Sprague-Dawley rats (castrated postnatal day 6) 42.43, treated postnatal 60-69). Exp. 3 evaluated the androgen antagonist flutamide (FT; 1, 5 or 10 mg/kg/day) in combination with MT (10 or 0 mg/kg/day). BT was administered p.o. first (ca. 8am) and MT was given p.o. ca. 4 hours later. Vehicle-dosed controls were included in all studies. Rats were euthanized 24 hours after the final dose. The liver, gallbladder (GBP), seminal vesicles (VP) and testes (fixed and fixed, seminal vesicles plus coagulating glands with sections (SGG), Cowper's glands (CG), and levator ani and bulbocavernosus muscles (LABC) were weighed to the nearest 0.1 mg. TP produced distinct dose-response in these tissues: dose (mg/kg) to produce maximum tissue weight: w.t.; Gp at 0.4 mg/kg/day; VP and CG at 0.8 mg/kg/day; LABC at 1.6 mg/kg/day, and SGG response was linear over the dose range. Responses (fold increase in tissue wt. compared to control) at 1 mg/kg/day dose were: w.t. 1.5 mg/kg/day dose ranged from ca. 2 to 25 in the group of LABC-CG-VP, SGG, MT p.o. was somewhat less potent; significant dose responses; fold. increase in tissue wt. compared to control at MT 50 mg/kg/day dose ranged from ca. 1.6 to 10.4 in the order: Gp-LABC-CG-VP-SGG, and Coefficients of Variance of tissue wt. ranged from 2 to 51.5, LABC-VP, VP-AG). Activity of MT was reduced by combined treatment with FT. No major differences in Dose-Response curves were seen between VP or CG. This comparison of assay performance by the relevant oral route to the s.c. route supports additional validation by both routes.

1354

DETECTION OF ENDOCRINE EFFECTS: AN ENHANCED OECD TEST GUIDELINE 407: STUDY WITH METHYLASTEROSE


As part of a global OECD exercise, an enhanced version of the OECD TG 407 (28-day rat toxicity study) is being evaluated for detection of endocrine disrupters. A range of different molecules acting via different modes of action are included in the study; the effects of an androgen agonist (methyltestosterone) in male and female rats are reported here. Histors rats were administered methyltestosterone by gavage 0, 10, 40 or 200 mg/kg/day in males and 0, 10, 100 or 600 mg/kg/day in females for at least 28 days. Endocrine-related parameters included in the study were: necropsy of females in diuresis only; assessment of sperm parameters (number, motility and morphology); determination of plasma hormone levels (T4, T3, TSH, LTH, testosterone and estradiol). A significant decrease in body weight gain (BWG) was observed in high dose male whereas all female treated groups showed a marked increase in BWG. Male rats at 40 and 200 mg/kg/day showed decreased testis weights and a low number of Leydig cells with diffuse degeneration of germinal epithelium associated with an alteration in the sperm parameters. In addition, prostate weights were increased in high dose males only. Females at 100 and 600 mg/kg/day showed gonadal atrophy (interstitial cells atrophy, follicular atresia) with epithelium hyperplasia of the uterus and vagina. Histopathological findings in both males and females were also observed in the liver, thyroid, adrenals, mammary glands and kidney. Hormonal measurements showed increased TSH and decreased LTH plasma levels in both sexes. These results support that a potent androgen can be detected in the enhanced version of the OECD TG 407. (Supported by EMSG and OECD)

1355

ASSESSMENT OF A FATHEAD MINNOW REPRODUCTION ASSAY FOR IDENTIFYING ENDOCRINE-DISPRACTING CHEMICALS WITH DIVERSE MODES OF ACTION


The USEPA has developed a short-term reproduction test with the fathead minnow to identify potential endocrine disrupting chemicals (EDCs). The assay is initiated by collecting baseline spawning data from reproductively-active adult fathead minnows for 21 days, followed by a 21-day exposure to sublethal concentrations of test materials. Measurements are made of fecundity, fertility, hatch success, secondary sex characteristics, gonadal condition (e.g., histopathology), plasma vitellogenin and plasma sex steroids (estradiol, testosterone, and 11-ketotestosterone). To evaluate performance of the assay, we have conducted tests with several chemicals representative of major modes of action of concern from an EDC perspective. Chemicals tested have included: methoxychlor (metabolites are estrogen receptor agonists); ZM189, 154 (estrogen receptor antagonist); methyltestosterone and trenbolone (androsterone receptor agonists); flutamide and vinclozolin (androsterone receptor antagonists); and fadrazole cyrochrome P450 aromatase [CYP19] inhibitor). Reductions in fecundity were clearly evident in exposures to methoxychlor, methyltestosterone, trenbolone, flutamide and fadrazole. These reproductive effects were accompanied by changes in other parameters reflective of the expected mode of action. For example, in the fadrazole test, estradiol and vitellogenin levels were decreased in females, as would be predicted if CYP19 was inhibited. Likewise exposure to the androgen receptor agonists caused masculinization in females, while estrogenic chemicals produced feminization (e.g., vitellogenin production) in males. This assessment of the test method shows the utility of the assay to provide guidance in identifying diverse modes of action of potential EDCs. This abstract does not necessarily reflect EPA policy.

1356

DEVELOPMENT OF AN AMPHIBIAN-BASED ASSAY TO SCREEN FOR CHEMICAL EFFECTS ON THE THYROID AXIS


In response to the initial EDSTAC recommendations, research was conducted on the development of a Xenopus laevis based tadpole resorption assay for evaluating thyroid axis disruption. The results of these initial experiments highlighted key limitations associated with relying on tail resorption as a measure of anti-thyroid activity. The most critical being that the tail tissue of tadpoles in metamorphic climax are innervated by perturbation by agonists and antagonist. To begin to improve on the initial proposal we have conducted experiment comparing the sensitivity of metamorphic (stage 51) and pro-metamorphic (stage 54) tadpoles to the model.
thyroid axis antagonists methimazole (control, 6.25, 12.5, 25, 50, 100 mg/L) and 6-propylthiouracil (control, 1.25, 2.5, 5, 10, and 20 mg/L). Tadpoles were exposed for a 2 wk. period, and developmental stage, thyroid size, and thyroid histology were examined at 1 and 2 wks after exposure. Methimazole treatment resulted in a dose-dependent inhibition of metamorphosis at both developmental stages within one week, indicating thyroid interaction in stage 51 tadpoles within one week and of both stages within two weeks. Further, both compounds caused dose-dependent changes in thyroid gland morphology. These changes were characterized as reduced colloid, glandular hypertrophy, and cellular hyperplasia. Treatment failed to affect growth, even in tadpoles which experienced significant metamorphic inhibition. As determined from these endpoints, there were only minor differences in sensitivity observed among the 2 stages examined. These results indicate that tadpoles in the early stages of metamorphosis are sensitive to thyroid axis inhibition and that development of a short term, diagnostic amphibian-based thyroid screening assay shows considerable promise. This animal does not necessarily reflect EPA policy.

1357
INTERPRETING THYROID TOXICITY FOR RISK ASSESSMENT.

R. T. Zoller.
Biology, University of Massachusetts, Amherst, MA.
Thyroid hormone is essential for normal brain development. A large number of chemicals, of both natural and synthetic origin, are known to affect thyroid function. Therefore, it is important to characterize the effects of specific chemicals on the ability of thyroid hormone to exert its action(s) during brain development. However, the present strategy for testing thyroid toxicity in rodent models employs only endpoints of thyroid function only—chemicals are not tested for their ability to interfere with thyroid hormone action. This strategy is valid if and only if chemicals interfere with thyroid hormone action exclusively by affecting thyroid function, or that measurable changes in thyroid function are always associated with changes in thyroid hormone action. Considering that the consequences of thyroid hormone deficits during development are not remediable, and that thyroid hormone action is quite pleiotropic, end points of thyroid hormone action must be reviewed and studied to be used for risk assessment. However, these measures must be identified and validated.

1358
EVALUATION OF A CANNULATION MODEL AND ISOFLURANE ANAESTHESIA FOR BLOOD SAMPLING IN THE RAT: EFFECTS ON SERUM PROLACTIN AND CORTICOSTERONE.

Toxicology and Pathology, Janssen Research Foundation,Beerse, Belgium. Sponsor:W. Powers.
The purpose of this study was to validate two different techniques for blood sampling in female Wistar Hannover rats, i.e. by permanent cannulation of the vena femoralis and by orbital puncture after isoflurane anaesthesia. To this purpose the effects of stress caused by the two techniques on two stress-sensitive hormones were measured, i.e. prolactin and corticosterone. Decapitation was used as a reference technique as it is believed to have minimal effects on these hormones. It is regarded by the American Veterinary Medical Association as an acceptable technique when properly performed (2000 report). Blood was sampled in all rats 7 days after surgery of the cannulated animals over a period of 4 hours at 4 timepoints. Repeated blood sampling was performed in ten rats while with the other techniques six animals were used on each timepoint. No in-life technical problems were encountered with any of these techniques although in cannulated animals the amount of blood that can be sampled is limited and sometimes insufficient. Both isoflurane anaesthesia and cannulation induced higher prolactin levels than decapitation and isoflurane anaesthesia induced higher prolactin levels than cannulation. Isoflurane anaesthesia and decapitation were comparable when corticosterone levels were measured. Cannulation induced higher corticosterone levels than both other techniques. Cannulation and isoflurane anaesthesia are technically feasible but have the potential to induce alterations in the levels of one or both hormones. Decapitation does not alter the levels of prolactin and corticosterone. Decapitation offers the possibility of repeated blood sampling in the same animal which reduces the intra-animal variability which can be useful for e.g. pharmacokinetic studies and PKPD studies.

1359
NADPH OXIDASE: VERSUS MITOCHONDRIAL OXIDANT STRESS IN ACETAMINOPHEN TOXICITY.

Previous studies have demonstrated that nitrooxygen (NT) adducts in proteins are formed in the centrilobular regions of the livers of mice dosed with acetaminophen (APAP). NT is formed by peroxyxynitrite, a species produced by the rapid reaction of superoxide and nitric oxide. Previous studies indicated that pre-treatment of mice or rats with macrophage inactivators resulted in decreased toxicity and NT formation. The initial site of nitroration was hepatic sinusoids. These data suggested a role for activated Kupffer cells in peroxynitrite formation. In the present study, we investigated the importance of superoxide generated by NADPH oxidase (present in Kupffer cells and neutrophils) in the development of APAP hepatotoxicity. APAP toxicity was compared in genetically engineered (knock out, KO) mice deficient in the gene encoding gp91phox, a large critical subunit of NADPH oxidase, and C57BL/6j (wild type, WT). Immunohistochemical analysis indicated a centrilobular distribution of APAP protein adducts and NT in both groups of mice. Sinusoidal staining for APAP and NT was present at 1 hour and subsequently hepatocyte staining for APAP and NT was observed in both groups of mice. These data indicate that superoxide from NADPH oxidase does not significantly contribute to peroxynitrite formation in APAP toxicity. However, peroxynitrite formation in sinusoidal endothelial cells and mitochondrial oxidant stress are important events in acetaminophen toxicity.

1360
MEMBRANE ASSOCIATION OF MURINE GSTA4-4, A GLUTATHIONE TRANSFERASE THAT ACTS ON LIPID PEROXIDATION PRODUCTS.

"Univ. of Arkansas for Medical Sciences, Little Rock, AR. Univ. of Texas Medical School, Houston, TX. Univ. of Texas Medical Branch, Galveston, TX and Univ. of Texas at Arlington, Arlington, TX.
Reactive oxygen species (ROS) react with proteins, nucleic acids, and lipids. The latter process produces lipid hydroperoxides and electrophilic α,β-unsaturated aldehydes including 4-hydroxyhexenal (4-HNE). The conjugation of 4-HNE with glutathione is catalysed by specialized α-class GSTs exemplified by murine mGSTA4-4. The crystal structure of mGSTA4-4 reveals the presence of two highly exposed lysine residues (K115, one per subunit). Position 115 is occupied by acidic residues in most other alpha-class GSTs. We hypothesized that the positive charges of K115 residues in mGSTA4-4 may interact with negative charges on membranes. This could "dock" the enzyme to membranes, i.e., the site of generation of its substrates (lipid peroxidation products). Therefore, we examined the intracellular localization of mGSTA4-4 in hepatocytes of normal mouse liver and in transfected HepG2 cells by fluorescence microscopy and digital deconvolution. mGSTA4-4 was found to be predominantly localized at or near the plasma membrane in transfected HepG2 cells, as well as in hepatocytes where it is expressed endogenously. mGSTA4-4 is associated with liposomes, and this interaction was potentiated when the liposomes contained phosphatidylserine. Mutating lysine-115 to glutamic acid resulted both in a loss of the plasma membrane targeting of mGSTA4-4 and in a significant reduction of its binding to liposomes in vivo. These data suggest that lysine-115 is critically important for the membrane association of mGSTA4-4, most likely by entering into an electrostatic interaction with negatively charged phospholipid headgroups, including phosphatidylserine. Membrane association of mGSTA4-4 may facilitate the transfer of lipid peroxidation products from the membrane to the active sites of the enzyme.

1361
GENERATION AND CHARACTERIZATION OF A KNOCKOUT MOUSE LACKING GSTA4-4. A GLUTATHIONE TRANSFERASE THAT METABOLIZES LIPID PEROXIDATION PRODUCTS.

M. R. Engle, J. D. Ceci, Y. C. Awasthi and P. Zimmick.
"Univ. of Arkansas for Medical Sciences, Little Rock, AR and University of Texas Medical Branch, Galveston, TX.
Lipid peroxidation products have signaling functions and, at high concentrations, are toxic and may trigger cell death. The murine α-class glutathione S-transferases exemplified by mGSTA4-4, an enzyme highly efficient in glutathione conjugation of 4-hydroxynalkenals, and possessing glutathione peroxidase activity toward phospholipid hydroperoxides. Metabolism of lipid peroxidation products may thus have a dual role: signal termination and detoxification. To determine the physiological implications of these processes, we generated a mGSTA4-4 knockout mouse by homologous recombination. The mouse is viable, although preliminary observations on a limited number of available animals suggest lower fertility. Northern and Western blotting confirmed the
1362 CYTOSKELETON FOLLOWING THE INDUCTION OF ENDOPLASMIC RETICULUM STRESS PROTEINS IS CELL TYPE AND TOXIN DEPENDENT.


Recent evidence suggests that induction of endoplasmic reticulum (ER) stress proteins increases cellular tolerance to agents which cause cytotoxicity through either oxidative stress or covalent binding. Previous work has been done primarily in kidney and neuronal cell types. To determine if protection against cytotoxic agents following the induction of endoplasmic reticulum (ER) stress proteins is a phenomenon that can be generalized across cell types, we assessed the protective effect of an ER stress response in a human hepatocyte cell line (HePG2), a rat hepatocyte cell line (H4IIE), a porcine kidney cell line (LLC-PK1), and a human lymphocyte cell line (K562). The ER stress response was induced by pretreatment with the glycanosylation inhibitor, tunicamycin. Induction of the ER stress proteins GRP78, GRP94 and protein disulfide isomerase (PDI) was assessed by immunoblotting. In all cells, at least two of the ER stress proteins were induced, although the pattern varied between cell types. Tolerance to 3 hour challenges with the alkylating agent indomethacin (IDAM), the oxidative stressors tert-butyldihydroperoxide (TBHP), the redox cycling agent menadione, or a 2 hour challenge with the reactive intermediate of acarnitinephin NAPQI was assessed by the MTT assay 24 hours after addition of the toxin. In LLC-PK1 cells, the dose of toxin resulting in 50% survival increased for IDAM (505%+115%), TBHP (1662%+58%) as previously reported, and for menadione (117%+9.0%) and NAPQI (205%+45%). However, in the hepatocyte and lymphocyte cell lines, increased tolerance was observed only in the HepG2 cells, where after ER stress, cells were more tolerant only to IDAM, exhibiting an 69% at a 8.8% increase in the TDS0 after tunicamycin treatment. Induction of ER stress proteins in H4IIE cells and K562 cells did not alter sensitivity to any toxins tested in those cells. Our results indicate that protection afforded by the induction of an ER stress response is dependent on the cell type and toxin.

1363 ENGINEERING OF A BINARY TRANSGENIC MOUSE BROADLY EXPRESSING FPG FOR TESTING THE PATHOLOGICAL ROLE OF OXIDATIVE DNA DAMAGE.

D. Huang1, J. T. Henderson2 and P. G. Weil3. 1Faculty of Pharmacy, Univ. of Toronto, Toronto, ON, Canada; 2Samuel Lunenfeld Research Inst., Mt. Sinai Hospital, Toronto, ON, Canada and Dept. of Pharmacology, Univ. of Toronto, Toronto, ON, Canada.

Reactive oxygen species (ROS) are formed endogenously, enhanced by some xenobiotics, and are believed to promote a number of pathological processes. ROS enhance the formation of 8-oxoguanine (8-oxo-G), a promutagenic oxidative DNA lesion that underpins base excision repair in mammalian mt 8-oxoguanine glycosylase (OGG1). OGG1’s bacterial counterpart is formamidopyrimidine DNA glycosylase (fpg). FPG exhibits an 80-fold higher activity and a broader substrate specificity compared to OGG1. To determine the role that oxidative DNA damage and repair plays in ROS-mediated toxicity, we engineered tetracycline-regulable transgenic mice that broadly expressed fpg during development. To optimize tetr regulated expression in these mice, transactivator (beta-actin promoter / TTA) and operator (Teo/tp) constructs were simultaneously introduced into murine embryonic stem (ES) cells. The resulting ES clones were selected for those with optimal performance characteristics, which were used to construct murine lines. To more easily detect fpg in vivo, a FLAG epitope was fused in-frame with our fpg cDNA. In addition, TetO /beta-Gal was introduced into some ES cell clones to allow for lac-Z and neomycin detection in affected cells. Prior to the introduction into ES cells, constructs were tested in HEK 293 cells. In these cells carboxy-terminal FLAG-tagged fpg was correctly localized to the cell nucleus, although it exhibited lower repair activity (~50%) compared to our wild-type fpg clones. Co-transfection of actin/TTA strongly activated expression of both Teo/tp/FLAG and TetO/beta-Gal as determined by Western blotting and lacZ cell staining. Transgene expression was ablated when cells were cultured in the presence of doxycycline. We are currently assessing the ROS response of our ES cell clones in response to various xenobiotic agents (Support: Canadian Institutes of Health Research).

1364 MUTATION OF THE TSC-2 GENE IN EKER RATS IS ASSOCIATED WITH REDUCED EXPRESSION OF 8-OXOGUANINE-DNA GLYOSYLASE (OGG1) AND A LACK OF INDUCTIBILITY DURING OXIDATIVE STRESS.

S. L. Habih, H. S. Yoon, T. L. Meek and S. S. Law. Center for Molecular and Cellular Toxicology, University of Texas at Austin, Austin, TX.

Treatment of either wild type (Tsc-2+/+) Eker rats, or those carrying the mutant Tsc-2 allele (Tsc-2-/-) with 3, 5-tris(glutathionyl)hydroquinone (TGQH; 2.5 mmol/kg, i.p. 4, 8 or 16 wk), a potent nephrotoxic metabolite of HQ, causes cell necrosis and proliferation within the outer stripe of the outer medulla (OSOM) in both groups of rats. However renal cell carcinomas only arise in the OSOM of the Tsc-2-/- strain. These findings provide compelling evidence that cell proliferation per se is not sufficient for tumor development. TGQH can redox cycle and generate reactive oxygen species, and the mutation spectra induced by TGQH following treatment and replication of the supF gene in both human AD293 cells and Escherichia coli MBL50 cells is consistent with that reported for OH- induced mutations. 8-Oxo-deoxoguanosine (8-oxoG) is not only a measure of oxidative stress, but is stress and a potentially mutagenic lesion. TGQH (2.5 mmol/kg, i.p.; 5X/wk for 4, 8, 16 wk) increased 8-oxoG-2 fold within the OSOM of both rat strains at 8 wk. While elevated 8-oxoG levels were sustained in Tsc-2-/- rats, they returned to baseline levels in Tsc-2+/+ rats at 16 wk. Interestingly, although OGG-1 expression in Tsc-2+/+ rats increased significantly during treatment with TGQH, no such induction occurred in Tsc-2-/- rats. Furthermore, the constitutive expression of OGG-1 was 3 fold lower in Tsc-2+/+ compared to Tsc-2-/- rats. These results suggest that mutation of the Tsc-2 gene is coupled to the suppression of constitutive OGG-1 expression and interferes with the ability to up-regulate this gene following oxidative stress. Moreover, the high basal and inducible OGG-1 levels in the wild type Eker rats likely provide for a more efficient removal of oxidative DNA damage in these rats, thereby limiting tumor development. The basis for the lack of OGG-1 induction in Tsc-2-/- is unknown and is currently being investigated. (GM339583, ES07784).

1365 COVALENT MODIFICATION OF PEPTIDES BY 4-HYDROXYNONENAL AND 4-OXONONENAL.

J. A. Dowen1 and D. B. Peterson2. 1Pharmacology, University of Colorado Health Sciences Center, Denver, CO and 2Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO.

Previous work has demonstrated that the alpha., beta-unaturated aldehydes 4-hydroxynonal (4HNE) and 4-oxononal (4ONE) are generated during lipid peroxidation. Recent evidence has implicated that covalent modification of proteins by 4HNE may play a role in some pathophysiological processes. Currently, the reactivity of 4ONE toward proteins is unknown. The purpose of this study was to identify residues that react with these alpha-, beta-unaturated aldehydes, characterize the covalent adduct(s) formed, and measure the kinetics of addition. Model peptides containing one or more susceptible residues (i.e., Lys, Met, Lys, Arg, and His) were reacted with a 20-fold excess of 4HNE and 4ONE at pH 7.4 and 37 °C. Control and treated peptides were analyzed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and post-source decay (PSD) analysis was used to confirm the presence of a peptide adduct and identify the residue modified. Bimolecular rate constants for the reaction of glutathione (GSH) with 4HNE and 4ONE were measured. Peaks with mass corresponding to the peptide with a Michael adduct were observed in the mass spectra of model peptides containing either Cys, His, Lys or Arg that had been treated with 4HNE. The rate constants for the reaction of GSH with 4HNE and 4ONE were found to be 1.33 and 143 M⁻¹s⁻¹, respectively, indicating a >100-fold difference in potency for GSH modification between these two compounds. The results demonstrate that both 4HNE and 4ONE react with nucleophilic peptide residues to form covalent adducts but with contrasting specificity and a remarkable difference in potency for GSH conjugation. (Supported by grants NIH/NIAAA R01AA93050, NIH/NEINS R01ES09410 and PHS 5T32AA07464-25).
1367 INCREASED HEPATOTOXICITY OF ACETAMINOPHEN IN TUMOR NECROSIS FACTOR ALPHA (TNF-α) KNOCKOUT MICE IS ASSOCIATED WITH DECREASED ACTIVATOR PROTEIN-1 (AP-1) ACTIVATION.

H. Chiou, C. R. Gardner, S. G. Rickets, J. D. Laskin, and D. L. Laskin, Joint Graduate Program in Toxicology, Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine implicated in the hepatotoxicity of acetaminophen. In the present studies, we examined possible mechanisms mediating the actions of TNF-α. Treatment of wild type control mice with acetaminophen (300 mg/kg, i.p.) resulted in a time-dependent induction of centrilobular hepatic necrosis and increased serum transaminase levels, which reached a maximum after 18 hours. Knockout mice lacking TNFR1 (p55) were found to be significantly more sensitive to acetaminophen than wild type mice, as determined histologically and by increases in serum transaminases. This was correlated with decreased levels of the antioxidants, superoxide dismutase and heme oxygenase, which correlated with 50% of wild type mouse characteristics. These data suggest that signaling pathways regulating the production of antioxidants were altered in TNFR1 knockout mice, we analyzed hepatic levels of p47-Rel N-alpha terminal kinasises (JNKs) and its downstream target, AP-1. Western blot analysis revealed that administration of acetaminophen to wild type mice resulted in a time-dependent activation of JNK, which was evident within 3 hours. Nuclear binding activity of the transcription factor, AP-1, measured by gel shift assays, was also increased. These responses were significantly reduced in mice lacking TNFR1. These data suggest that TNF-1 plays an important role in the activation of JNK and AP-1, as well as in increases in antioxidants during acetaminophen-induced hepatotoxicity. Supported by NIH GM34310 and E096897.

1368 INDUCIBLE NITRIC OXIDE SYNTHASE KNOCK-OUT (iNOS(-/-)) MICE EXHIBIT INCREASED OXIDATIVE STRESS INJURY IN RESPONSE TOADRIMACIN (ADR)-INDUCED CARDIOTOXICITY.

M. P. Cole, T. D. Oberley, S. M. Lin, K. K. Kinningham and D. K. St. Clair, 'Graduate Center for Toxicology and Nutritional Sciences, Univ. of KY, Lexington, KY and Dept. of Pathology, Univ. of WI, Madison, WI.

ADR is a chemotherapeutic agent known to cause dose-related cardiotoxicity. Heart injury associated with ADR treatment involves the generation of free radicals, such as superoxide. Increases in superoxide in the presence of reaction metal leads to the generation of the injurious hydroxyl radical. Alternatively, superoxide can react with nitric oxide (NO) forming peroxynitrite. Therefore, the levels of NO can alter the fate of superoxide radicals in vivo. Here we investigate the role of NO in ADR-induced cardiotoxicity using an iNOS(-/-) mouse model. Electron microscopy demonstrated that hearts following 5 days following one 3 mg/kg peritoneal injection of ADR (20 mg/kg), iNOS(-/-) mice exhibit cytoplasmic swelling and degradation of mitochondria as compared to wildtype (iNOS(+/-)) mice. Tissue damage, assessed by creatine phosphokinase, lactate dehydrogenase, and cardiac troponin serum levels, is increased in iNOS(-/-) mice as compared to iNOS(+/-) mice, supporting the pathological findings. Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are increased to a larger degree in iNOS(-/-) mice than iNOS(+/-) mice following ADR, indicating a compromise in their ability to illicit an adaptive response to ADR-induced oxidative injury. The oxidized to reduced glutathione ratio increases in iNOS(-/-) mice treated with ADR and an increase in 4-hydroxynonenal (4-HNE) adducts is observed 24 hours following treatment. Further verifying an increase in oxidative stress in the knock-out model. Time course studies indicate an increase in nitrotyrosine prior to the increase in 4-HNE in iNOS(-/-) mice, but not in iNOS(+/-) mice. Endothelial NOS protein levels are unchanged following treatment with ADR in both iNOS(-/-) and wildtype mice. These findings suggest that ADR-induced NO production is an early cardioprotective event associated with adaptation to oxidative stress.

1369 OXIDATIVE STRESS CAUSES THE FORMATION OF TWO DISULFIDES IN HUMAN THIOREDOXIN.

W. H. Waszon, J. Puhl, O. Stuchlik and D. P. Jones, Biochemistry, Emory University, Atlanta, GA.

Thioredoxin (Trx) is a small protein that restores disulfide bonds in proteins to the reduced form following oxidative stress. Trx contains two essential active site cysteines that cycle between the dithiol and disulfide forms as Trx reduces target proteins. Human Trx contains three additional cysteines, the functions of which are unclear. The purpose of the present study was to determine the redox state of human Trx under defined redox conditions and before and after oxidative stress within cells. Purified Trx was reduced with DTT or oxidized with diamide or hydrogen peroxide, derivatized with iodoacetate which carbamoylated thiols but not disulfides, and separated by non-reducing, non-denaturing polyacrylamide gel electrophoresis. MALDI-TOF mass spectrometry of tryptic digests of individual gel bands demonstrated unequivocally the locations of carbamoylated cysteines and disulfide bonds. Human Trx treated with diamide or hydrogen peroxide contained two disulfide bonds: one between Cys32 and Cys35 (the active site cysteines), and one between Cys62 and Cys69. To define the midpoint potentials, Trx was equilibrated in glutathione redox buffers. The midpoint potential for the active site disulfide, determined by plotting the ratio of the oxidized and reduced forms of Trx against the potential of the rox buffer, was -230 mV. The second disulfide bond formed only under more oxidizing conditions, with a midpoint potential of about -150 mV. Within cells, the redox state of Trx was -280 mV, and the two-disulfide form of Trx was not detected. However, Trx was rapidly oxidized to the two-disulfide form upon exposure of the cells to t-butyldihydroperoxide. Thus, human Trx contains a second cisthol that undergoes oxidation during oxidative stress. The function of this non-active site disulfide is unknown, but its presence near the interface region suggests that it may regulate redox-dependent interactions with other proteins.

1370 INVOLVEMENT OF THE ELECTROPHILE RESPONSIVE ELEMENT (ERE) IN THE ACTIVATION OF HEPATIC STELLATE CELLS DURING FIBROGENESIS.

L. Qamar, A. Pappa, D. P. Purnell and V. Vanguri, Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO.

Hepatic stellate cells (HSC) produce the type I collagen in normal and cirrhotic liver. During liver fibrogenesis HSC undergo an activation process that includes increased proliferation and synthesis of extracellular matrix proteins, along with a phenotypic transformation to myofibroblastic-like cells. Activated HSC contributes to chronic hepatic inflammation, which ultimately leads to cirrhosis. We have found a 20-fold increase of NAPDH:quinone oxidoreductase 1 (NQ01) and quinone oxidoreductase 2 (NQO2) activity and protein levels in a HSC line derived from cirrhosis (CFSC) rat liver compared to that of normal (NFSC) rat liver. This NQ01 up-regulation appears to be responsible for the resistance of the CFSC line cells to menadione cytotoxicity. Gel mobility shift assays and functional analysis using transient transfection experiments suggest that the involvement of the Electrophile Responsive Element (ERE) in the up-regulation of the NQ01 gene expression in CFSC cells. In conclusion, the EPRE-signaling pathway, which regulates several antioxidant genes, may play a major role in the resistance of HSC against oxidative stress that occurs during hepatic fibrogenesis. This EPRE-mediated resistance may be part of the HSC activation process, which takes place during liver injury. [Supported by NIH RO1 AA18858 & RO1 09300-05].

1371 OLIGONUCLEOTIDE MICROARRAY ANALYSIS REVEALS AN ANTIOXIDANT RESPONSIVE ELEMENT-DRIVEN GENE SET INVOLVED IN CONFRERING PROTECTION FROM AN OXIDATIVE STRESS-INDUCED APOPTOSIS IN IMR-32 HUMAN NEUROBLASTOMA CELLS.

J. Li, J. M. Lee and J. A. Johnson, UW School of Pharmacy, Madison, WI. Sponsor: J. Johnson.

The current study was aimed to investigate whether and how t-butyldihydroperoxide (tBHQ) prevents hydrogen peroxide-induced apoptosis in neuroblastoma cells. Hydrogen peroxide at a final concentration of 200 μM induced IMR-32 cells...
to undergo apoptosis with morphological changes such as condensed and fragmented nuclei (TUNEL assay), increased caspase-3 activation, and poly ADP-ribose polymerase cleavage. In cytotoxicity assays, both TUNEL assay and 5-H2B peroxidase treatment (10 μM) attenuated hydrogen peroxide-induced cell death and the number of TUNEL-positive cells, respectively. We hypothesized that 5-H2B-mediated activation of the antioxidant response element (ARE) is critical for generating the protective response. Our laboratory has recently shown that a selective inhibitor of phosphorylatedinositol 3-kinase (PI3-kinase), LY294002, blocks ARE activation by 5-H2B. Addition of LY294002 30 min prior to 5-H2B treatment completely reversed the protective effect of 5-H2B. Oligonucleotide microarray were used to analyze the gene expression profile associated with 5-H2B treatment in the absence and presence of 30 μM 5-H2B. Our laboratory has recently shown that a selective inhibitor of phosphorylatedinositol 3-kinase (PI3-kinase), LY294002, blocks ARE activation by 5-H2B. Many of these genes function to combat oxidative stress and increase the cells' deoxygenation potential through increased production of NADPH, glutathione (reduced form), and ATP. Some example are NADPH quinone oxidoreductase, gamma-glutamylcysteine ligase regulatory subunits, thioredoxin reductase, glutathione reductase, and cytosolic malic enzymes. Inhibition of PI3-kinase significantly blocked the enhanced expression of 49 of the 63 genes induced by 5-H2B. These are the first data to show the gene set involved in conferring protection from an oxidative stress-induced apoptosis and imply that the PI3-kinase-Nrf-2 ARE pathway is critical in mediating protection from oxidative stress in human neuroblastoma cells.

1372
IS THERE A RELATIONSHIP BETWEEN URINARY AND LUCOFUCYTE 8-HYDROXYDEOXYGUANOSINE IN HUMANS?
M. Torason, P. Mathias, A. Ruder, M. A. Butler, L. Taylor, C. Forrest, E. Krieg, and D.G. Delbridge. NIOSH, Cincinnati, OH. OH

8-Hydroxydeoxyguanosine (8-OHdG) is typically measured in DNA of white blood cells (WBCs) or in urine. Urine measurement is believed to provide an index of oxidative DNA damage repair. WBCs are considered a surrogate for target tissues. These markers are used almost interchangeably to assess the adverse effects of a variety of environmental factors. Only a few of the hundreds of studies assessing 8-OHdG as a biomarker of oxidative DNA damage in humans, have measured 8-OHdG in both urine and WBC DNA. Two recent studies reported non-significant inverse associations between urinary excretion of 8-OHdG and WBC DNA 8-OHdG. We examined this relationship in male roosters with (n=26) or without (n=12) exposure to polycyclic aromatic compounds and female dry cleaners and launderers with (N=18) and without (N=20) exposure to perchloroethylene. For males, urine and blood were obtained pre-shift at the start and post-shift at the end of a work week. For females, urine was obtained post- and post-shift mid-work week, and blood was obtained once pre-shift and mid-work week, 8-OHdG was measured by HPLC-ECD. Linear models were used to look for relationships between leukocyte DNA and urine levels of 8-OHdG. Terms for gender, exposure, smoking, and race were included. Interaction terms were also included to test if the relationship between blood and urine levels varied by these terms. The models were also used to estimate and test individual slopes. For the model comparing pre-shift urine with post-shift, no main effects or interactions were statistically significant, but a significant inverse association (p = 0.023) was observed in males (r = -0.36, p < 0.036) and was noted for the individual sub-group of female controls. For the model comparing pre-shift urine with post-shift urine, no main effects or interactions or individual slopes were statistically significant. For the model comparing post-shift blood to post-shift urine, the smoking interaction was significant (p = 0.0314). Results demonstrate a limited association between leukocyte DNA 8-OHdG and urinary excretion of 8-OHdG.

1373
EFFECTIVE FACTORS ON URINARY 1-HYDROXYPYRENE IN A KOREAN POPULATION.
M. Yang, S. Lee, D. Kang, H. Shin, S. Kim and J. Jang. Preventive Medicine, Seoul National University, Seoul, South Korea. and Preventive Medicine, Ajou University, Suwon, South Korea.

Urinary 1-hydroxypyrene (1-OHP) has been used as a biomarker for exposure to environmental carcinogens like polycyclic aromatic hydrocarbons (PAHs). For proper biological monitoring of the PAH exposure, associated factors with 1-OHP bioproduction should be clarified. In this study, we investigated effects of lifestyle, environmental factors, and genetic polymorphisms of metabolic enzymes, i.e., GSTM1, GSTT1, CYP1A1 and CYP1B1 on urinary 1-OHP levels in 700 Korean population (male, 65%; female, 35%; mean age, 36 yrs and 10.79 yrs) who were not occupationally exposed to PAHs. Using questionnaire, we obtained information of cigarette smoking, indoor heating-system, house-environment, consumption of well-dose meat, vegetables, yogurt, coffee, alcohol, etc. We analyzed urinary 1-OHP and cotinine, a biomarker of smoking, by HPLC. To determine genotypes of the enzymes, we used PCR-RFLP and single base extension methods.

As results, urinary 1-OHP was detected in 76% of the subjects (range, 0.1-3.8 μg/L). In the Korean population, CYP1B1 codon 48 and 119 polymorphism were completely linked and CYP1B1 codon 48 polymorphism was associated with codon 432 polymorphism (p < 0.05). Urinary 1-OHP was also increased with urinary cotinine level, number of cigarette smoked before sampling, yogurt consumption, and GSTT1 polymorphism (Spearman Rank correlation, p < 0.05). However, polymorphisms of GSTM1, CYP1A1, and CYP1B1 were not associated with urinary 1-OHP levels. After multiple regression analysis, urinary 1-OHP was associated with only number of cigarettes smoked before sampling (p < 0.05). In conclusion, recent cigarette smoking affects urinary 1-OHP compared to any other environmental, food- or genetic factors.

1374
NEUROPATHY TARGET ESTERASE (NTE) IN WHOLE BLOOD: BIOMARKER FOR EXPOSURE TO NEUROPATHIC ORGANOPHOSPHORUS COMPOUNDS (OPs). G. F. Makhaeva, V. I. Sigacheva, L. V. Zhusovets, A. V. Ereminets, I. N. Kurochin, R. J. Richardson and V. V. Malysin. Institute of Physiologically Active Compounds Russian Academy of Science, St. Petersburg, Russian Federation. Center of Molecular Diagnostics and Therapy, Moscow, Russian Federation and Toxicology Program, Michigan University.

NTE is the target protein for neuropathic OPs that cause OP-induced delayed neurotoxicity (OPIDN). The inhibition of aging of brain NTE within hours of exposure predicts the potential for the development of OPIDN in susceptible animal models. Lymphocyte NTE has also found limited use as a biomarker of human exposure to neuropathic OPs. Recently, we have developed a highly sensitive biosensor for NTE assay using a tyrosinase carbon-paste electrode to detect phenol produced by the hydrolysis of the substrate, phenyl valerate. The biosensor enabled NTE to be assayed in whole human and hen blood, whereas the usual colorimetric assay is impossible. NTE activity in hen and human blood was found to be 0.16 ± 0.03 and 0.19 ± 0.02 nmol/min x mg of protein, respectively. Mipafox, Iq, values for hen and human blood NTE were found to be 4.22 ± 0.12 and 6.27 ± 0.43 μM, respectively. To study the possibility of using blood NTE inhibition as a biochemical marker of neuropathic OPs exposure, NTE inhibition in brain and lymphocytes as well as in blood and brain was studied 24 hr after dosing hens with the neuropathic OP O,O-dipropylthiodichlorovinyl phosphates. Brain, lymphocyte and blood NTE were inhibited in a dose-responsive manner. There were strong correlations of NTE inhibition between brain and lymphocyte, brain and blood, and lymphocyte and blood. Taking into account the small sample volume required, simplicity of sample preparation, rapid analysis time, stability of samples after freezing, and strong correlation of NTE inhibition between blood and brain, our biosensor NTE assay for whole blood shows promise, not only as a biomarker of human exposure to neuropathic OP compounds, but also as a predictor of OPIDN and an adjunct to its early diagnosis. Supported by ISTC, Project 1055.2.

1375
CHOLESTEROL ESTERASE ACTIVITY IN MICE CHRONICALLY EXPOSED TO PYRIDOSTIGMINE BROMIDE. R. D. Grubbs, W. A. Pigg, B. S. Mauck, I. Bernovna, S. J. Paton, D. R. Cool, J. B. Lucot and M. Morris. Pharmacology & Toxicology, Wright State University, Dayton, OH.

Pyridostigmine bromide (PB), given to soldiers during the Persian Gulf War as a prophylactic against potential nerve gas attack, is now thought to be a possible causative agent of the symptoms of Gulf War Syndrome. We have developed a murine model to test the effect of chronic exposure to PB on cholesteryl esterase activity in the blood and the brain. C57Bl/6 mice were surgically implanted with osmotic mini-pumps to deliver a steady dose of PB over 7 days. ChE activity was determined by a modified version of the colorimetric assay of Elman, et al. Total ChE activity was measured in diluted whole blood and brain homogenate samples. Blood acetylcholinesterase (AChE) activity was determined by inhibiting butyrylcholinesterase (BChE) with iso-OMPA (tertisopropyliborphorhodanin). BChE activity was then calculated by subtracting AChE activity from total ChE activity. Following 7 days of exposure to PB, reductions of blood ChE activity of 27%, 42%, and 53% were seen in the 5, 10 and 40 mg/kg/D dosage groups, respectively, when compared in pretreatment levels. The decrease in blood ChE activity due primarily to a drop in AChE activity, indicated that the osmotic mini-pump delivery system was functioning properly. We found no change in ChE activity in sham-implanted control animals. Interestingly, while analysis of preferential correct showed no significant change in AChE activity after exposure to PB, we observed a dose-dependent decrease in AChE activity (48% at 3 mg/kg) in the hypoglycemic clamp of PB treated mice (see Ropp, et al. abstract, this meeting). We are currently evaluating ChE activity in other brain regions of these animals and other mice exposed to both chronic stress and PB. Our findings indicate that chronic exposure to PB pro-

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1376 EVALUATION OF OXIDATIVE PROCESSES IN T LYMHPOTYME PROLIFERATION AND DEATH WITH CYTOCHROME P450 INHIBITORS AND HIGH-SPEED CAPILLARY GC/TOFMS MEASUREMENT OF VOLATILE COMPOUNDS OF MONM. 

S. L. Berney and F. Ebisu, V. Venketashchalam, V. Bhaskara, H. Jayatilake and H. L. Holmen, Chemistry, Eastern Michigan University, Ypsilanti, MI.

A murine T cell hybridoma (2B4, 1.1) was used to investigate how oxidative processes contribute to cell proliferation and to cell death induced by various agents. Inhibitors of the oxygen-activating enzyme cytochrome P450 attenuated cell death caused by calcium ionophore or T cell receptor ligand, but not deamethasone, at concentrations that did not cause cell death. These results suggest that the P450 monooxygenase system plays a role in some pathways that signal T cell death. In view of the role of cytochrome P450 and other pro-oxidant enzymes in lipid peroxidation, methodology was developed to identify and measure biomarkers of lipid peroxidation in T cells. Malondialdehyde, as detected by the spectrophotometric measurement of the thiobarbituric acid complex, was produced in T cells during proliferation, was enhanced in the presence of 1 mM actinomycin D, and was inhibited by the addition of 20 µM ascorbate. To monitor the volatile products of lipid peroxidation, peri dishes containing T cell hybridoma cells were incubated at 37°C in a closed chamber equipped with a fan to disperse volatile products of cellular metabolism. The head-space gas was passed through a membrane (provided by Resek) to exclude water and was then collected and concentrated over a five-minute period at 30°C by a trap cooled by liquid nitrogen. Firing of the trap was achieved at 170°C by application of a 90-sec voltage. Using this novel method, hexanal was easily identified by GC/TOFMS above the 10 nM of about 0.5 × 10^6 T cells/mL in RPMI 1640/10% fetal bovine serum. The simultaneous characterization of volatile products in a continuous assay provides a non-invasive approach to examining the kinetics of formation of volatile lipid peroxidation products formed during oxidative stress induced by pro-oxidant systems such as cytochrome P450. [Supported by Research Excellence Fund/Graduate School Research Support Fund (EMU) and NSF Grant DBI-9987063].


We have evaluated whether serum calmodulin (CaM) can be used as a biological marker in chronic lead exposure. Thirty-six workers exposed to lead from a storage battery factory were selected as exposed group and twenty-six non-exposed control group. Workers were selected as control group. Controlling factors such as age, gender, length of service, marriage and bad habits were carefully considered in the evaluation. Blood CaM, blood lead, calcium, magnesium, copper and zinc were determined from both lead and non-exposed workers and all data were analyzed using SAS software. The blood CaM was reduced in workers with high blood lead levels. At blood lead level higher than or equal to 40 µg/dl; the blood CaM in exposed group was found to be (35.1±8.6) µg/dl, which was significantly lower when compared to the control group (75.6±10.35 µg/dl; p<0.05). Age, length of exposure, marriage status and bad habits had no effect on blood CaM levels (p>0.05). The correlation analysis results showed that blood lead and blood calcium was correlated to blood CaM levels (p<0.05), while blood magnesium, copper and zinc had no significant correlation with CaM (p>0.05). The results indicated that reduction of blood CaM in lead-exposed workers was accompanied by the increased blood lead. The concentration of 40 µg/dl might be considered as the biological threshold for blood lead, which coincided with the current diagnostic standard for blood lead in China. The results of the present study suggested and may indicate a role of CaM as a biological marker. However, further study with increased number of lead exposed individuals will be required before CaM could be suggested as a biological marker for chronic lead exposure.

1378 SIMULTANEOUS DETERMINATION OF N-7 HYDROXYETHYLGLUCINATE, O2- HYDROXYETHYLGLUCINATE AND N-3 HYDROXYETHYLADENINE WITH LC/MS/MS.

H. U. Kafferlein and T. R. Fennell. CIT Center for Health Research, Research Triangle Park, NC.

N-7-hydroxyethylguanine (7-HEG) has been previously described as the major DNA adduct in vivo after exposure to ethylene oxide (EO), with analysis by gas chromatography/mass spectrometry (GC/MS) or high performance liquid chro-

matography (HPLC) with fluorescence or electrochemical detection. Here we present a method for the determination of 7-HEG by HPLC and tandem mass spectrometry (LC/MS/MS). In addition, the method is also capable of analysis of O2- hydroxethylguanine (6-HEG) and N-3-hydroxyethyladenine (3-HEA) in one analytical run. For this purpose, DNA was isolated from mouse liver tissue by chloroform/pHENol extraction. DNA samples were fortified with the adducts mentioned above and subsequently, thermal hydrolysis was carried out. Separation of adducts from oligonucleotides was achieved by centrifugation of the hydrolysate using microconcentration filters. After injection and separation of the adducts employing a novel stationary phase (silica-bonded polysilane) for HPLC, the adducts were quantified by means of their isotopic labeling analogues N-7- and O2- hydroxethyl-[13C8]-, N-7- hydroxyethyl-[13C8]-, N-6-9-7-dihydroxyethyl[13C8]-6-HEG* and 6-HEG*, and N-3- hydroxyethyl-[13C8]-, N-6-9-dihydroxyethyl[13C8]-3-HEA*. Multiple reaction monitoring (MRM) was carried out to detect the fragments [M+44] for the analytes and their labeled internal standards. The limits of detection for the method were determined to be between 2.6 and 2.3 fmol on column depending on the particular adduct.

The LC/MS/MS method presented here has advantages over the previously published GC/MS methods, since derivatization of adducts could be omitted and all three major adducts of EO can be analyzed in one single analytical run.

1379 ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (ESI-MS/MS) ANALYSIS OF 1- BROMOPROPANE MERCAPTURIC ACID METABOLITES IN URINE.


1-Bromopropane (1-BP CAS 106-95-5), an alternative solvent to chlorofluorocarbons, has been reported to cause reproductive and neurotoxicity in male rats. The related 2-bromopropene has been shown to cause similar toxicity in rats and amenorrhea, oligospermatism, and amenorrhea in workers. Activation to reactive intermediates may be important. Metabolism of 1-BP is complex, including deamination, CYP2E1 oxidation and glutathione S-conjugation. The formation of 3-bromopropionic acid and n-propenal as well as Sn-propyl-glutathione, Sn-propyl-L-cysteine and the mercapturic acids N-acetyl-S-(n-propyl)-L-cysteine (M1), N-acetyl-S-(n-propyl)-L-cysteine-S-oxide (M2), N-acetyl-S-(2-carboxyethyl)-L-cysteine (M3) and N-acetyl-S-(3-hydroxy-n-propyl)-L-cysteine (M4) as potential biomarkers was developed to measure urinary levels of M1, M2, M3 and M4. Standards and a stable isotope-labeled analog of M1 as internal standard were synthesized using the general procedure of van Bladeren et al. (1980). Samples mixed with internal standard were loaded onto Bond Elute C18 SPE columns. A fraction containing >90% of 1-BP metabolites was eluted with acetonitrile for analysis by HPLC. ESI-MS/MS on a 150×2 mm Phenomenex Aqua C18 column using a 10-min H2O:MeOH (1:9% acetic acid) linear gradient at 300 µL/min. The ESI-MS/MS operated in the positive ion mode to detect protonated M1, M2, M3 and M4 and used Selected Reaction Monitoring of major transition products. Urine samples fortified with standards were mixed with 10 µg/mL internal standard and evaluated for recovery, limits of quantitation and calibration. Calibration of M1, M2, M3 and M4 was linear from 30 - 10000 mg/mL (r=0.999). Sample preparation and analysis appears to offer significant advantages over typical derivatization that would be required for GC-MS analysis of these compounds. Thus, 1-BP internal exposure levels for various exposure situations can be rapidly determined by analysis of these metabolites in a single assay using selective sample preparation.

1380 IMMUNOLOGICAL DETECTION OF HEXAMETHYLENEDIISOCYANATE ADDUCTS IN SERA FROM AUTOBODY SHOP WORKERS.

R. Lenus, L. Lukanovka and M. H. Kapl. Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA.

Hexamethylene diisocyanate (HDI), a potent irritant and allergen, is widely used in the manufacture of polyurethanes for paints and coatings. Detection of HDI in blood is problematic since diisocyanates are difficult to characterize, measure and control. Biomarkers would be of great benefit for early detection of exposure to prevent possible chronic adverse health effects. We have previously reported development of a sensitive and specific immunoassay for detection of HDI adducts on proteins. Microtiter plates coated with rabbit IgG from anti-HDI-keyhole limpet hemocyanin antiserum were used to capture HDI-adducted proteins. The immunoassay has a sensitivity of 95 nmol HDI adduct. To evaluate the potential use of the immunoassay to detect recent HDI exposure, sera were collected from six autobody shop workers who are exposed to HDI in paints. Serum samples were obtained from three of these workers periodically during one workday, and from the remaining three workers only at 24 hr after the start of the workday. Sera were also obtained from control non-exposed individuals. HDI adducts were detected using
peroxidase-labeled sheep anti-human albumin, followed by substrate. No HDI adducts were detected in any of the samples from control individuals nor in samples taken at the end of the workday. However, in one worker (397 nmol HDI adduct was detected at 4 hr; and 422 nmol was detected at 24 hr; HDI adducts were detected in sera from three of the individuals at 24 hr. Concentrations ranged from 104-426 nmol adduct. These results indicate that this immunoassay has appropriate sensitivity to detect recent HDI exposure and can be applied for human bio-monitoring of HDI in the workplace. Supported by NIEHS #5651.

1381 ACETAMINOPHEN PROTEIN ADDUCTS IN MOUSE LIVER AND SERUM AND HUMAN SERUM AFTER HEPATOPOISIC DIOXES OF ACETAMINOPHEN DETECTED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION.


Acetaminophen-induced hepatotoxicity has been attributed to covalent binding of the reactive metabolite N-acetyl-p-benzoquinone imine to cytosine groups on proteins as an acetaminophen-cysteine conjugate. We report a high-performance liquid chromatography with electrochemical detection (HPLC-ECD) assay for the conjugate with increased sensitivity compared to previous methods. Previous methods to quantitate the protein-bound conjugate have used a competitive immunosay, or radiolabeled acetaminophen. With HPLC-ECD, the protein samples are diazotized then digested with protease. The acetaminophen-cysteine conjugate is then quantified by HPLC-ECD utilizing tyrosine as an internal reference. Acetaminophen protein adducts were detected in liver and serum as early as 15 min after hepatotoxic dosing of acetaminophen to mice. Adducts were also detected in the serum of acetaminophen overdose patients. Analysis of human serum samples for the acetaminophen-cysteine conjugate revealed a positive correlation between acetaminophen-cysteine conjugate concentration and the serum aspirate amonotransferase (AST) and alanine aminotransferase (ALT) activities, and time to treatment with the antidote, N-acetylcysteine. Adducts were detected in the serum of patients even with relatively mild liver injury, as measured by AST and ALT. This assay may be useful in the diagnostic evaluation of patients with hepatotoxicity of an indeterminate etiology, for which acetaminophen toxicity is suspect.

1382 AN LC-ESI-MS/MS ASSAY FOR THE QUANTITATION OF CROTONALDEHYDE-DERIVED EXOCYCLIC 1, N² PROPAANOXYGOXANOSINE ADDUCTS.

M. D. Stout, A. L. Ham, H. Koc, R. Sangiash and J. A. Sweeney, Curriculum in Toxicology and Departments of Environmental Sciences and Engineering, and Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.

Crotonaldehyde is a genotoxic and carcinogenic cet, beta-unaturated aldehyde. Endogenous formation of crotonaldehyde presumably occurs through lipid peroxidation. Crotonaldehyde is a proposed metabolite of butadiene and is also ubiquitous in our environment due to automobile exhaust and industrial processes. Upon reaction with DNA, crotonaldehyde forms, among several other classes of DNA adducts, a pair of diastereomeric exocyclic 1, N²-propanoxygenosine adducts. These propanoxygenosine adducts are present in two cell systems in which crotonaldehyde is mutagenic. The ubiquity and mutagenicity of crotonaldehyde point to a need for the development of biomarkers for the assessment of crotonaldehyde exposure and risk. A sensitive and specific adduct quantitation method employing mass spectrometry would permit measurements of adducts at low doses and comparisons between endogenous and exogenous adducts. To this end, we have initiated development of an LC-ESI-MS/MS assay to quantitate crotonaldehyde-derived exocyclic 1, N²-propanoxygenosine adducts (C-Guo 1&2). Both analyte and [4, 5, 6, 8-¹³C] labeled internal standards for C-Guo 1&2 have been synthesized and characterized by HPLC, UV, full scan MS, and NMR. The first analyte standard was diazotized during HPLC has been analyzed by LC-ESI-MS/MS (SRM 338-222) to determine preliminary conditions for quantitation of these adducts and to determine their sensitivity to LC-ESI-MS/MS. LC-ESI-MS/MS has proven to be an extraordinarily sensitive method for detecting these adducts, as for the analysis of the standard yielded signal to noise ratios of up to 114. Method development is continuing with more sensitive LC-ESI-MS/MS work to optimize the detection of C-Guo 1&2 in DNA samples, and to determine a cleanup procedure for enrichment of C-Guo 1&2 in DNA samples. Following the completion of method development, we will quantitate C-Guo 1&2 in a variety of DNA samples.

1383 EFFECT OF CYP2E1 GENOTYPE ON ACYRONITRILE HEMOGLOBIN ADDUCTS.


Acrionitrile (ACN) is metabolized by two pathways: conjugation with glutathione, and oxidation to cyanohydrine (CE) by an epoxide and ACN metabolites indicated the absence of oxidation to cyanohydrine, and ACN adducts were isolated by solid phase extraction, and chromatographed by reverse phase HPLC. Elution was monitored by selected reaction monitoring on a PE Sci API 3000 triple quadrupole mass spectrometer. The method can measure adducts from natural abundance and stable isotope labeled ACN. To investigate the impact of the CYP2E1 genotype on ACN internal dose, [1, 2, 3, 4] ACN (20 mg/kg) was administered to wild type and CYP2E1-null mice, and blood was collected for CE analysis. In the wild type mice, CE levels were approximately 3 fold lower than in the CYP2E1-null mice (1487 ± 728 fmol/mg globin vs. 4863 ± 911 fmol/mg globin). These data indicate that, in the absence of CYP2E1 and the capability to oxidize ACN, the internal dose of acrylonitrile in the CYP2E1-null mice increased to greater than 3 times the levels in wild type mice.

1384 ESTIMATING POPULATION MEANS FOR THE DISTRIBUTIONS OF POLYCHLORINATED BIPHENYL CONGENERS IN CENSORED DATA SETS.


Samples of milk from 99 lactating women were analyzed for 99 PCB congeners, DDE, Mirex, and hexachlorobenzene (HCB) (Kostyniak et al. Environmental Research 80:166-174, 1999). Eight PCB congeners and one pesticide (DDT) were found in amounts above the limit of detection (LOD) in all 99 women. The remaining PCB congeners and pesticides were found in some, but not all, of the women. Values below the LOD are treated as censored data. Several methods have been suggested in the literature for estimating the mean and standard deviation of the population of concentrations for censored data sets. In the present study we examine the performance of five methods: substitution of the LOD or half the LOD (LOD/2), the maximum likelihood estimator (MLE), the restricted maximum likelihood estimator (RML) and a linear regression analysis (LR). We compare the estimators' performance in the eight PCBs and in DDE for which there is a complete data set. The mean and standard deviation are calculated for each congener of the full data set. Censored data sets were created from the complete data set by dropping the smallest 10, 20, 30, 40, 50, 60, 70, 80, and 90 percent of the data. The methods were then compared for each degree of censoring, using the percent error as a measure of the bias of the estimates of the mean, and the ratio of the observed to estimated variance of the population in the relative efficiency of the estimator. The RML, MLE and LR gave estimates of the mean within 10% of the actual value for all levels of censoring. Although we could not statistically distinguish between the RML, MLE and LR, all three methods performed significantly better than substitution of the LOD or LOD/2. In conclusion the RML, MLE and LR are more useful than substitution of the LOD or LOD/2 to estimate the population mean PCB congener concentration in censored data sets.

1385 SECULAR CHANGE OF BIOLOGICAL MONITORING MEASUREMENTS IN JAPAN.


Partial amendments to the Japanese Ordinance on the Prevention of Lead Poisoning and the Japanese Ordinance of the Prevention of Organic Solvent Poisoning were made in 1989. As a result, the measurement of lead in blood and urinary delta-amino-levulinic acid (ALA) became indispensable items of the occupational health examination for workers who handle lead. Also, the measurement of urinary metabolites of workers who handle eight kinds of organic solvents became mandatory. In order to support the national-wide biological monitoring program in Japan, seven major laboratories meet regularly to improve quality control and collect the measurement results after the enforcement of the amended ordinance. The annual number of measurements was about 100,000 for lead in blood and ALA in urine, and about 500,000 for the metabolites of the organic solvents, however, the total number of the subjects has slightly decreased for the last 10 years. The number of urine with high ALA concentration decreased drastically during the
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CHANGES OF URINARY AND BLOOD Porphyrins DURING PROLONGED EXPOSURE AND POST-EXPOSURE TO PCBs, PB OR DIAZINON IN RATS.


Porphyrins are intermediates in the biosynthesis of heme. The porphyrin pattern in excreta or urine has been introduced as a useful biomarker for hazardous xenobiotics. In this study, we evaluated and compared the urinary and blood porphyrin profiles by treatment of PCBs (Aroclor1254), PB or diazinon in rats. PCBs (10, 50, and 100 mg/kg bw), PB (62.5, 250, and 1,000 ppm) and diazinon (10, 30 and 90 mg/kg bw) were administered to SD male rats via gavage (PCBs and diazinon) or via drinking water (PB) daily for 5 weeks. The highest dose of each chemical was selected as LD10. Uro, copro and protoporphyrin in urine and blood collected every week for 5 weeks of exposure and 5 weeks of post exposure were quantified with fluorospectrometer. No clinical signs were observed by PB and diazinon, while icterus and 15% lethality were induced by 100mg/kg PCBs. Three of urinary porphyrins and blood protoporphyrin were increased gradually and dose responsively in progress of PCBs exposure time and then urinary coproporphyrin came to peak at 5 weeks of exposure, urinary uroporphyrin and blood protoporphyrin at 2 weeks after withdrawal and urinary protoporphyrin at 3 weeks after withdrawal. They were continued to be higher than control until 5 weeks after PCBs withdrawal. By the treatment of PB, the increase of urinary porphyrins and blood protoporphyrin was induced more rapidly than control, then decreased more quickly than PCBs. That is, urinary porphyrins was increased rapidly, came to peak at 2 weeks of exposure and returned to normal levels as soon as withdrawal and blood porphyrin began to increase from 1 week, peaked at 5 weeks of treatment and continued to be higher than control during withdrawal period. In diazinon case, urinary and blood porphyrins were not so much sensitively altered as PCBs and PB, just showing week increase of urinary coproporphyrin. These results demonstrate that urinary and blood porphyrin profiles confer characteristic biomarkers and useful tools for exposure assessment of PCBs, PB and diazinon.

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PLATINUM LEVELS IN NASAL LAVAGE ARE NOT RELATED TO TRAFFIC DENSITY BUT TO NASAL INFLAMMATION.

P. J. Born1, D. Polat1, J. Begerow1, M. Türefeld, A. Becker1 and R. R. Schin1.

1Particle Toxology Institut für Umweltmedizinische Forschung (IUF), Düsseldorf, Germany and Analytical Chemistry Medizinische Institution für Umweltforschung, Düsseldorf, Germany. Sponsor: P. Kallavanti.

Platinum (Pt) is a well-known constituent of particles emitted by auto catalysts. To evaluate Pt as a potential marker for traffic related particle exposure, we investigated Pt content along with non-traffic related metals (Cr, V) in coarse and fine particulate matter (PM) sampled in 4 areas with different traffic density as well as in nasal lavage fluid (NLF) of 63 children living in these areas. Pt, Cr and V content of coarse and fine PM were not significantly different between different sites, although CO emission as a validated traffic indicator during the sampling interval was clearly different (ANOVA, P < 0.05). In addition, concentrations of these metals in nasal lavage of children living in these locations were not significantly different. However, a significant correlation between Pt levels in NALP and total number of cells in the blood for the inflammatory status of the nose was found. No relationship was present between inflammation and Cr and V levels in nasal lavage. We suggest that Pt is a marker for traffic-related PM that are able to induce inflammation in the upper respiratory tract.

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USE OF DNA ARRAYS TO MONITOR GENE EXPRESSION IN BLOOD AND UTERUS FROM LONG-EVANS RATS FOLLOWING 17 BETA-FASSTRODIOL EXPOSURE - A NEW APPROACH TO BIOMONITORING ENDOCRINE DISRUPTING CHEMICALS USING SURROGATE TISSUES.


Evidence exists that environmental xenobiotics are disrupting the endocrine systems of certain wildlife species, resulting in developmental abnormalities and reproductive impairment. Although the relationship between human disease and exposure to such endocrine disrupting chemicals (EDCs) is unknown, it is believed that EDCs present a potential threat to public health. Gene expression analysis offers the ability to identify preclinical exposures to EDCs and provide mechanistic data useful for risk assessments, and could ultimately provide a vehicle for developing early diagnostic and preventative measures in at-risk populations or individuals. Since apparently healthy human reproductive organs cannot be sampled directly, a method to utilize more accessible tissues must be developed. We propose that gene expression changes in accessible tissues such as blood will often reflect those in inaccessible tissues, thus offering a convenient biomonitoring method for providing insight into the effects of environmental toxicants on target tissues. In this pilot study, gene expression changes in peripheral blood lymphocytes (PBLs) were compared with those in the uterus of adult rats to identify genes that were altered in both tissues following estradiol treatment. Overactinized rats were treated with either 17-beta-estradiol or vehicle control for three days. Uteri and PBLs were harvested, and total RNA extracted and hybridized to Clonartich Atlas rat 2.1 arrays. A number of genes were found to demonstrate a similar degree of expression change that was tissue-, but not site-specific, demonstrating that accessible tissues such as PBLs can act as surrogate tissues for observing gene expression changes in inaccessible target tissues.

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PLATELET MAO-B ACTIVITY AS A STATE MARKER OF ALCOHOLISM.

L. Manna1, C. Gandini1, A. F. Castoldi1, G. Randone1 and T. Coccini1.

1Toxicology Division, Maggiore Foundation, Pavia, Italy and 1Dep. of Internal Medicine, University of Pavia, Pavia, Italy.

This study evaluated time-related changes in platelet monoamine oxidase B (MAO) activity in a homogeneous Italian cohort of alcoholic-dependent subjects (n = 98) during early abstinence and the effect of potential confounding factors, such as gender and smoking status, on the temporal trend of this enzymatic activity. While under observation of abstinence (baseline point T1) MAO activity in alcoholics was 6.4 ± 3.1 nmol/mg protein/hr. This returned within the control range (9.9 ± 0.9 nmol/mg protein/hr) after 8 days of withdrawal (T2), and remained stable thereafter (T3 and T4: 15 and 22 days of abstinence, respectively). Alcohol intake decrease was confirmed by serum %carbohydrate-deficient transferrin (CDT), which was pathologically above the reference limits (6%) at (T7: 1.8 ± 3.9%), decreased to 6.6 ± 2.1% at T2 and reached physiological values at T3 and T4. In cirrhotic alcoholics %CDT did not decrease over time, while MAO activity was comparable to that of abstinent noncirrhotic alcoholics. The mean values of other liver function tests were elevated at the first evaluation (GGT: serum gamma-glutamyltransferase: 64 ± 894 U/I; AST: aspartate aminotransferase: 113 ± 70 U/I; ALT: alanine aminotransferase: 86 ± 56 U/I), decreased during hospitalization but remained above the reference values at the patient's dismissal (GGT: 231 ± 487 U/I; AST: 30 ± 34 U/I; ALT: 47 ± 37 U/I). MCV (mean corpuscular volume) values were elevated at admission and remained so during hospitalization. Neither gender nor tobacco smoking affected the temporal pattern of MAO activity during early withdrawal. MAO-B can be regarded as a state marker of alcohol consumption. The temporal pattern of its activity may be used for the diagnostic assessment of alcoholism and early abstinence, regardless of gender and smoking status. (Supported by grant QLRT-2001-00186 from the European Commission).

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CYTOCHROME OXIDASE ACTIVITY AND CHOLESTEROL MUSCARINIC RECEPTOR BINDING AS PERIPHERAL MARKERS OF CARBON MONOXIDE NEUROTOXICITY.

T. Coccini1, A. F. Castoldi1, C. Gandini1, G. Randone1 and L. Manna1.

1Toxicology Division, Maggiore Foundation, Pavia, Italy and 1Dep. of Internal Medicine, University of Pavia, Pavia, Italy.

Carbon monoxide (CO) is a widely spread neurotoxicant, has been shown to alter cytochrome oxidase C (COX) activity and cholesterol parameters in the brain of acutely exposed rodents. A decrease in COX activity has also been observed in the lymphocytes of acutely poisoned humans. In this study we investigated the early and delayed effects of in vitro CO exposure on cholesterol muscarinic receptor (MR) binding and COX activity in the rat brain and whether CO-exposed central changes would be reflected by similar alterations in peripheral lymphocytes. Male Wistar rats were exposed by inhalation to 500 ppm CO for 6 hr/day, 5 days/wk for 4 wks. One and 7 days after the end of the treatment, no changes were detected in 3H-QNB binding to MR in the cerebral cortex, cerebellum and hippocampus (control values, mean ± SD, were for cerebral cortex, cerebellum and hippocampus 171 ± 45, 77 ± 7 and 245 ± 53 fmol/mg tissue, respectively) as well as in the lymphocytes (24 ± 19 fmol/million cells). Similarly ineffective was repeated CO exposure (towards brain (124 ± 25 nmol/min/mg protein) and lymphocyte (26 ± 9 nmol/min/mg protein) COX activity at the same points. Acute CO inhalation (2400 ppm, 1 hr) did not modify central and peripheral COX activity and MR
binding 1 hr, 1 day, 3 and 7 days after treatment. Studies on other possible CO targets (such as cGMP) are in progress to order to identify valuable peripheral biomarkers of central effect (Supported by grant QL4K-CT99-01536 from the European Commission).

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SENSITIVE EARLY INDICATORS OF HEPATIC AND KIDNEY DAMAGE IN WORKERS EXPOSED TO JET FUEL [P-8].

J. F. Stawicki, M. A. Butler, J. C. Clark, E. A. Knecht and E. F. Krieg. *Biological Monitoring Laboratory Service, NIOSH, Cincinnati, OH.*

Jet Propellant type 8 (JP-8) is used for all US military operations. Because of the widespread exposure and concern for potential health effects, three hundred twenty-four US Air Force (USAF) active duty personnel participated in a study at six USAF bases between April and September, 2000. Animal & human studies suggest that exposure to fuels and solvents can adversely affect the liver and kidney. Glutathione transferase (GST) isoforms possess specific patterns of distribution in organs and are released to biological fluids in response to toxic insult. ELISA techniques can be used to measure those GSTs associated with liver and kidney damage. Pre- and post-shift serum and urine samples were collected from USAF personnel and commercial GST assays were used to measure alpha GST in serum (marker for liver damage) and alpha and pi GST in urine which are associated with proximal and distal tubule damage, respectively. Exposed workers were tank-entry personnel with at least nine months of persistent exposure to jet fuel; the unexposed group was USAF personnel with no significant exposure to fuels or solvents. Levels of serum hepatic alpha-GST and urinary nphritic alpha- and pi-GST in the study subjects fell within the normal range for healthy subjects. No differences were observed indicative of liver or kidney damage attributable to any of the exposure, lifestyle and other demographic variables examined. Creatinine to normalize urinary GSTs was elevated in post-shift samples from the highest exposure category. This study group represents a very healthy segment of the population. Sensitive measures for liver and kidney damage did not detect any adverse effects in this study group. Evidence of elevated creatinine in the mean post-shift samples of the high exposure category was seen. However, while these values are within normal clinical ranges, they are consistent with concentrated urine indicative of mild dehydration.

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A BIOLOGICAL LIMIT VALUE (BLV) FOR OCCUPATIONAL EXPOSURE TO PERFLUOROCANOATE.

R. Rey, G. Olsen, P. Lieder, J. Mandel and J. Buttenholf. *Medical Departments, 3M Company, St. Paul, MN.*

A biological limit value (BLV) of 5 ppm for perfluorocanoate (PFOA) in the serum of workers has been established by 3M. Ammonium PFOA (APFO) is a polymerization aid used in fluoropolymer production. The 3M BLV represents the best scientifically-based estimate of a concentration of PFOA in a biological fluid (serum) that, if present, even on a chronic basis, is not expected to correlate with a risk of adverse health effects in workers. Serum levels above the 5 ppm BLV do not necessarily imply a health risk. A significant body of toxicological, epidemiological, and medical surveillance information is available for PFOA and APFO to support a BLV of 5 ppm PFOA in serum. The results of toxicology studies indicate that the major target organ of APFO is the liver. Oral administration of APFO to monkeys at 3 and 10 mg/kg/day for six months resulted in mean serum concentrations of approximately 50 and 70 ppm, respectively, and was associated with increases in both absolute liver weight and liver to body weight ratio. These findings are consistent with histopathological abnormalities or changes in clinical chemistry. Approximately 90 percent of 3M workers exposed to APFO have had serum PFOA levels below 10 ppm. Investigators have not observed any exposure-related clinical hepatic toxicity or hormonal changes in these workers. The repeated analyses, to date, provide reasonable assurance that workers with serum PFOA levels below 10 ppm do not experience exposure-related clinical hepatoxicity; however, there have been too few employees with serum PFOA concentrations above 10 ppm to draw statistically-significant conclusions regarding a lack of clinical hepatoxic effects with serum PFOA levels at or above 10 ppm.

1393
DIFFERENT RESPONSES IN THE PATTERN OF EXPRESSION OF P53 CHALLENGED WITH ARSENIC.


Different pattern of expression of the p53 protein were observed when lymphocyte cultures were challenged with arsenic. To evaluate the reproducibility of this effect we determined the presence of p53 in blood samples from 22 healthy individuals, using 4 different treatment conditions: (1) non-stimulated lymphocytes (G0); (2) lymphocytes stimulated with phytohemagglutinin during 48h (Control); (3) stimulated lymphocytes treated with arsenic(III) or (4) with actinomycin-D (Acd) during the last 24h. Proteins were obtained at the end of culture, and were measured by immunoblot. Show data three different patterns of p53 expression: 1) 16 individuals had bands in As and Acd treated cultures but not in G0; II) 4 individuals in addition to the previous pattern, showed a band in G0; III) 2 donors did not show any p53 response. No relation of these expression-pattern differences with cell proliferation could be established. Since frequency found of polymorphicisms in the intron 1 (8 Ig II P53) among breast cancer patients, we analyzed the p53 expression in females with and without polymorphism. No relationship between both parameters was found. The expression of p53 in lymphocyte cultures could be of relevance in the understanding of individual susceptibility to xenobiotics and might have a great potential as a biomarker.

1394
BIOMARKER CHARACTERIZATION OF ASPHALT FUME EXPOSURE.


Each year more than 60 million tons of asphalt are produced and used worldwide. It has been estimated that approximately two million workers were employed and potentially exposed to asphalt. Health concern from exposure to asphalt is related to its content of hazardous chemicals, such as polycyclic aromatic hydrocarbons (PAHs). The development of a biomarker to provide an assessment of the integrated external exposure following uptake by living system through inhalation is valuable for this kind of complex mixture exposure. Many PAHs are metabolized to hydroxy-PAHs and excreted in urine; therefore, urinary hydroxy-PAH was used as a biomarker for asphalt fume exposure in this study. Female Sprague-Dawley, DDY, and 6C3F1 mice were exposed to asphalt fume in a whole body inhalation chamber for 5 or 10 days (3.5 hrs/day, 71-97 mg/m³). Clean air chamber animals were used as controls. The test asphalt was the type used by paving industry throughout the Midwestern United States. The fume was generated under simulated road paving conditions. Urine sample preparation for the analysis of hydroxy-PAHs metabolites included enzymatic digestion, solid phase extraction, and isolation dilution GC/MS and microflow LC-Q-TOF MS detections. Four priorities PAH metabolites, including, 1-hydroxypyrene, 9-phenanthrene, 1-chrysene, and 2-naphthol were identified and determined in urine. Isotopoe-isopolar has proved to be useful for quantification of those metabolites. Preliminary results indicated that urinary PAH metabolite could be a sensitive biomarker following asphalt fume exposure. The method developed in this study has potential application to monitor of PAHs from occupational mixture exposure.

1395
BIOMARKERS OF OXIDATIVE STRESS STUDY: ARE OXIDATION PRODUCTS OF LIPIDS MARKERS IN CCL4 POISONING?


Oxidation products of lipids in plasma and urine of rats were measured as part of a comprehensive, multilaboratory validation study searching for non-invasive biomarkers of oxidative stress. This is the second report of the nationwide Biomarkers of Oxidative Stress Study (BOSS). The time (G, 7, and 16 h) and dose (120 and 1200 mg/kg ip) dependent effects of CCl4 on concentrations of lipid hydroperoxides, TBARS, malondialdehyde (MDA) and isoprostanes were investigated with different techniques. Plasma concentrations of MDA and isoprostanes (measured by GC/MS) and urinary concentrations of isoprostanes (measured with an immunoeassay) were increased in CCl4 treated rats in a time- and dose-dependent manner. All other products were not changed by CCl4 or showed only high-dose and/or single time point effects. It is concluded that measurements of MDA and isoprostanes concentrations in plasma and urinary isoprostanes are promising candidates for general biomarkers of oxidative stress. Acknowledgements: B Ames, N Brot, G Fitzgerald, R Floyd, M George, G Hanch, J Heineke, K Henson, J Lawson, J Larnett, J Morrow, D Murray, J Plasater, L J Roberts, M Shigenaga, R Sohal, Jie Sun, R Tice, D H Van Thiel, D Weller, P Walter, Children's Hospital Oakland Res. Inst., Oakland, CA, 4 Hospital for Special Surgery and Department, Microbiol, Immunol, Weil Med. College of Cornell University, NY, NY, 5 Center for Exp. Therapeutics, University, Pennsylvania, Philadelphia, PA. 6 UMR, Oklahoma City, OK. 7 Loyola University Med. Center, Maywood, IL. 8 USEPA, Research Triangle Park, NC, 9 Department, Mol. Biol, Pharmacology, Washington University, School of Medicine, St. Louis, MO, 10 Department, Biochem, School of Medicine, Vanderbilt University, Nashville, TN, 11 Department, Pharmac and Medicine, Vanderbilt University, Nashville, TN, 12 Otis Inc., Portland, OR, 13 Department, Biol.
1396 URBINARY BIPHENOL A GLUCURONIDE AS A BIOMARKER FOR BIPHENOL A EXPOSURE.


The estrogenic effects of bisphenol A (BPA) have been reported in E-screen assay and in vivo studies of rodents; however, the exposure level and health effects of BPA in humans are still unknown. We assessed the BPA exposure by the measurement of urinary BPA-glucuronide. University students and workers who handled BPA diacylglycerol ether (DGEBA-BPA) were evaluated in the present study. There urinary BPA levels were measured using a HPLC with fluorescence detector. BPA-glucuronide concentration was calculated by the subtraction of BPA measurement without β-glucuronidase treatment from BPA measurement with β-glucuronidase treatment. The method developed for urinary BPA determination shows a linear calibration, and good recovery rates and reproducibility. The urinary BPA levels of the students in 1992 were significantly higher than those of 1999, and BPA levels in the students who habitually drink more coffee and tea tended to be higher than those of students who drink less. The urinary BPA levels of BPA-exposed workers were significantly higher than those of the control workers. It is thought that the high levels of urinary BPA of the students in 1992 were caused by drinking BPA dissolved from inside-wall coating of coffee and tea cans. However, our recent BPA exposure seems to be very low because the inside coatings had been changed. Although the workers who handle BPA have a risk of BPA exposure, their exposure levels were almost same as those of the university students in 1992.

1397 RAPID GENOTYPE METHODS TO DISTINGUISH SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN THE HUMAN NAT1 AND NAT2 GENES.

M. A. DOLL and D. W. HEC. Pharmacology and Toxicology, University of Louisville, Louisville, KY.

N-acetyltransferase 1 (NAT1) and 2 (NAT2) catalyze the activation and deactivation of aromatic amine drugs and carcinogens. Genetic polymorphisms in NAT1 and NAT2 have been associated with the incidence of cancers related to exposures to aromatic and heterocyclic amines found in cooked foods, cigarette smoke, and the environment. We designed facile NAT1 and NAT2 genotyping methods that distinguish among the most frequent and functional nucleotide polymorphisms in the NAT1 and NAT2 coding regions and NAT1.3*uncalculated region (UTR). The single nucleotide polymorphisms detected in NAT1 are: C977T (R33Stop), C196T (R64W), G459A (V149D), G595T (R187Stop), G560A (R187Q), A752T (D251V), T1088A (3'UTR), C1095A (3'UTR). All NAT1 alleles are: NAT1.3, NAT1.4, NAT1.10, NAT1.11B, NAT1.11, NAT1.14, NAT1.14B, NAT1.15, NAT1.17, NAT1.19, NAT1.22 except very rare ones are detected by this method. The single nucleotide polymorphisms detected in the NAT2 genotyping method are: G191A (R64Q), C282T (silent), T341C (I114T), C481T (silent), G590A (R197Q), A805G (N268R), G857A (G286T). This method can detect all except very rare human NAT2 alleles including NAT2.5A, NAT2.5B, NAT2.5C, NAT2.5D, NAT2.5E, NAT2.5F, NAT2.5G, NAT2.5H, NAT2.5L, NAT2.5M, NAT2.5N, NAT2.5O, NAT2.5P, NAT2.5Q, NAT2.5R, NAT2.5S, NAT2.5T, NAT2.5U, NAT2.5V, NAT2.5W, NAT2.5X, NAT2.5Y, NAT2.5Z. These genotyping methods do not require post-PCR processing or the use of radioactivity, and therefore are suitable for high-throughput applications. Partial support by USPHS grant CA-34627 from the National Cancer Institute.

1398 COMPARING THE EFFECTS OF ALTEPLASE (TPA), TENECTEPLASE (TNK-TPA) And RETEPLASE (RPA) ON HEMOSTATIC PARAMETERS IN A RABBIT MODEL OF PROLONGED INFUSION.


Urokinase unavailability has led clinicians to use other thrombolytics for catheter-directed thrombolysis (CDT) in treating acute limb ischemia and deep vein thrombosis. Despite the widespread clinical use of thrombolytics, there is no predilection safety data evaluating the effects of these agents on coagulation parameters when administered using CDT. This study evaluated the effect of tPA, TNK-tPA, or rPA on hemostatic parameters using a rabbit model and infusion techniques similar to clinical practice. tPA, TNK-tPA, or rPA were infused (IV) at doses corresponding to 0.5, 1.0, 2.0, and 10 mg/kg (tPA), TNK-tPA, U/D (rPA) in a 70 kg human. Arterial blood samples were collected at 0, 0.5, 1, 2, 3, and 4 hours for coagulation parameters (PT, aPTT, fibrinogen, plasminogen and alpha2-antiplasmin). A dose related change in PT and systemic fibrinogen was exhibited with all thrombolytics; however, rPA had a greater increase in PT and greater depletion of systemic fibrinogen than tPA or TNK-tPA. rPA showed reduced alpha2-antiplasmin depletion (>50% of baseline at all doses) compared to TNK-tPA (20% of baseline with 0.143 mg/kg TNK-tPA) and rPA (0.40 and 0.50% of baseline with 0.143, 0.209 and 0.014 U/kg rPA, respectively). Plasminogen levels exhibited similar dose related changes. This data indicates significantly less alteration of systemic hemostasis parameters using highly fibrin-specific agents, tPA and TNK-tPA, compared to rPA. Consequently, tPA and TNK-tPA may provide a better safety profile with less systemic fibrinolysis when used for peripheral vascular applications; further comparative studies are warranted.

1399 EARLY EFFECT OF PHOSPHODIESTERASE (PDE) IV INHIBITION ON LEUKOCYTE-ENDOTHELIAL CELL INTERACTION: FUNCTIONAL RELEVANCE OF ENHANCED EXPRESSION OF INTEGRINS AND ADHESION MOLECULES TO EXPERIMENTAL VASCULITIS IN RATS.


PDE inhibitors have been shown to cause vasculitis in nonclinical toxicity studies, typically in rats. Vasculitis, such as that induced by the type IV PDE inhibitor CI-1018 is characterized by moderate to severe lesions with perivascular mixed inflammatory cell infiltrates. Underlying mechanisms associated with induction of the vascular inflammatory process are poorly understood. In the present studies we investigated the ability of CI-1018 to modulate early events of leukocyte-endothelial cell interactions, leukocyte surface expression of beta2-integrins, and gene regulation of the corresponding endothelial counter ligand intercellular adhesion molecule-1 (ICAM-1). Leukocyte adhesion and rolling activity were studied in female Wistar rats by intravital microscopy (IVM) between 1.5 and 4 hours following a single oral dose of CI-1018 at 750 mg/kg. IVM revealed an increase in the number of adherent and rolling leukocytes in mesenteric postcapillary venules. In a separate experiment, flow cytometric analysis showed that neutrophil surface integrin expression was upregulated 4 hours after a single dose of CI-1018. Using an in vitro leukocyte-endothelial adhesion assay, significantly more neutrophils from CI-1018-treated rats adhered to TNF-alpha-activated cultures of rat endothelial cells compared to controls. Blockade with anti-CD18 antibody significantly decreased neutrophil adhesion to the endothelial cell monolayer. These results suggest that CI-1018 induces early leukocyte adhesion which is mediated, at least in part, by beta2-integrin and the endothelial ligand ICAM-1. These effects likely have functional relevance to the pathogenesis of PDE IV inhibitor-induced vascular inflammation in rats.

1400 EXPRESSION OF ENDOTHELIAL AND INDUCTIVE NITRIC OXIDE SYNTHASE AND FORMATION OF PEROXYNITRITE-MODIFIED PROTEINS IN PHOSPHODIESTERASE (PDE) IV INHIBITOR-INDUCED VASCULITIS IN RATS.


The type IV PDE inhibitor CI-1018 has been shown to cause vascular lesions in nonclinical toxicity studies, typically in rats. It has been proposed that excessive release of nitric oxide by inducible nitric oxide synthase (iNOS) and subsequent formation of reactive species plays a role in tissue damage in PDE inhibitor-induced vascular injury. This study was conducted to determine the purative role of the cellular source of iNOS, to identify the distribution of nitrotyrosine residues as a measure of peroxynitrite production, and to evaluate their association with vascular injury induced by CI-1018. Four groups of female Wistar rats were given doses of vehicle or CI-1018 at 750 mg/kg daily by gavage for up to 3 days. One group of CI-1018-treated rats was sacrificed at 24 hours after each dose. Endothelial nitric
oxide synthase (eNOS), iNOS and nitrotyrosine were evaluated by immunohistochemistry in paraffin sections of mesenteric artery. Arteries showed intense iNOS immunostaining of endothelial cells in rats that received the full course of 3 daily doses of CI-1018 and only in sections that showed mild to marked vascular injury. eNOS was evident in endothelial cells without differences in staining intensity between control and CI-1018-treated groups. Nitrotyrosine-positive cells were detected only on reactive oxygen species-producing inflammatory cells, distinct from iNOS-producing endothelial cells. Nitrotyrosine-positive staining was present exclusively in inflammatory cells at 72 hours, coinciding with the peak in iNOS expression in mesenteric artery. It is concluded that PDE IV inhibitor-induced vascular injury in rats is associated temporally with the induction of iNOS in endothelial cells and with production of peroxynitrite by inflammatory cell infiltrates, but direct evidence of peroxynitrite-mediated vascular injury remains to be demonstrated.

1401 EFFECTS OF VARIOUS COMPOUNDS ON ACTION POTENTIAL PARAMETERS IN ISOLATED DOG PURKINJE FIBERS.


The QT interval on the electrocardiogram is the time from the onset of ventricular depolarization (the Q wave) to completion of repolarization (the end of the T wave) in the axis of the lead chosen for measurement. The QT interval is of special interest in view of potential therapeutic agents that can influence the duration of the interval and the known association between QT prolongation and the potential for malignant ventricular arrhythmias. As part of preclinical safety assessment packages, both in vivo and in vitro systems can be used to demonstrate effects of chemicals on electrophysiological and electrocardiographic parameters. In vitro Purkinje fiber preparations from a variety of laboratory species are considered to be suitable experimental models provided that the major ion current underlying the investigated cardiac action potential do not differ substantially from those encountered in human cardiac tissues. In this study, the effects of various compounds on action potential parameters were determined in isolated canine cardiac Purkinje fibers using intracellular microelectrodes. Action potential parameters measured were diastolic membrane potential, maximum rate of depolarization of action potential upstroke, overshoot action potential, action potential amplitude and action potential duration at 30, 50 and 90% repolarization. Reference standards of dl-sotalol and 1-phenylephrine were observed to prolong action potential duration, while lidocaine was found to shorten action potential duration. Lidocaine shortened action potential duration at all concentrations and frequencies tested. Interestingly, the effects of the 1-phenylephrine standard on action potentials were found to be time-dependent and biphasic. Low concentrations of phenylephrine rapidly prolonged action potential duration, but the maximum extent of this prolongation was not maintained throughout the 30 min exposure period. The standard dl-sotalol was observed to prolong action potential duration in a

1402 PRE-Clinical ASSESSMENT OF CARDIOtoxicity AND IN COMMON MAMMoSETs.

K. Kuswano1, R. Nagata2, S. Nagayama1, T. Hamada3, T. Kamenosono2, K. Fukushima2 and G. Kizaki2. Shin Nippon Biomedical Laboratories (SNBL), Ltd., Kayoshima, Japan and SNBL USA, Ltd., Everett, WA.

Appropriate species selection for safety pharmacology and drug toxicity testing is essential for the development of novel pharmaceuticals for human use. The purpose of this study was to establish a framework for conducting cardiovascular safety assessments in commonly used mammalian species. In this study, we have evaluated the electrophysiological effects of 5 anti-arrhythmic agents that have been approved for clinical use. Drug administration was performed under standard conditions and at the maximum dose levels tested. The drug dose was chosen to produce at least 25% prolongation of the QT interval in a reference standard (cloned 1-phenylephrine) for each species. The results of this study indicate that there is a significant correlation between in vivo and in vitro measurements of the QT interval. Additionally, there was a consistent relationship between drug-induced prolongation of the QT interval and clinical safety profiles. These findings suggest that in vivo electrophysiological measurements can be used to predict clinical safety profiles in a variety of species.

1403 INDUCTION OF ANTIOXIDANTS BY 3H-1, 2-DITHIOHE-3-THIONE IN CARDIOVASCULAR CELLS AFFORDS PROTECTION AGAINST OXIDATIVE CELL INJURY.

Y. Li, Z. Cao and M. Trush. Department of Pharmaceutical Sciences, St. John's University College of Pharmacy, Jamaica, NY and Division of Toxicological Sciences, Johns Hopkins University School of Public Health, Baltimore, MD.

Considerable evidence suggests that reactive oxygen species (ROS) are critically involved in the pathogenesis of cardiovascular diseases, particularly atherosclerosis. Consistent with this concept, consumption of antioxidants, such as vitamin E, has been shown to be causally related to a decreased incidence of atherosclerosis. However, whether induction of endogenous cellular antioxidants by chemicals (drug) also affords protection against oxidative cardiovascular injury has not been extensively investigated. In this study, using rat aortic A10 cells as an in vitro system we have characterized the induction of cellular antioxidants by the antioxidan induceing agent, 3H-1, 2-dithiole-3-thione (DST) and the protective effects against ROS-mediated injury in cardiovascular cells. Incubation of A10 cells with DST for 24 hours resulted in a significant dose-dependent induction of a battery of cellular antioxidants, including reduced glutathione (GSH), GSH peroxidase, glutathione reductase, catalase, and superoxide dismutase. To further examine the protective effects of the induced endogenous antioxidants against oxidative cell injury, A10 cells were pretreated with DST and then exposed to xanthine oxidase (XO) plus xanthine, a system that produces superoxide and hydrogen peroxide. We observed that DST pretreatment of A10 cells led to significant protection against XO/xanthine-induced injury as determined by 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) reduction and morphological changes. Taken together, this study demonstrates that a number of endogenous antioxidants in smooth muscle cells can be induced by exposure to DST, and that this chemical induction of cellular antioxidants is accompanied by markedly increased resistance to ROS-mediated cardiovascular cell injury.

1404 CHARACTERIZATION OF CELLULAR GLUTATHIONE AND GLUTATHIONE S-TRANSFERASE, AND THEIR INDUCIBILITY BY 3H-1, 2-DITHIOLE-3-THIONE IN RAT AORTIC SMOOTH MUSCLE A10 CELLS: PROTECTION AGAINST REACTIVE ALDEHYDE-INDUCED TOXICITY.

Z. Cao, M. Trush and Y. Li. Department of Pharmaceutical Science, St. John's University College of Pharmacy, Jamaica, NY and Division of Toxicological Sciences, The Johns Hopkins University School of Public Health, Baltimore, MD.

There is increasing evidence that aldehydes generated endogenously during the degradation process of biological molecules are involved in the pathogenesis of cardiovascular diseases, such as atherosclerosis. Because glutathione (GSH) and GSH S-transferase (GST) are major cellular defenses against the toxic effects of reactive aldehydes, in this study we have characterized the inducibility of GSH and GST by 3H-1, 2-dithiole-3-thione (DST) and their protective effects against acrolein and 4-hydroxy-2-nonenal (4-HNE)-induced toxicity in rat aortic smooth muscle A10 cells. Incubation of A10 cells with micromolar concentrations of DST resulted in a concentration- and time-dependent induction of both GSH and GST. Furthermore, the levels of GSH and GST remained higher than basal levels 72 hours following DST from the culture media. To examine the protective effects of DST-induced GSH and GST against reactive aldehyde-mediated toxicity, A10 cells were pretreated with DST and then exposed to acrolein or 4-HNE. Pretreatment of A10 cells with DST resulted in a marked decrease of acrolein or 4-HNE-induced toxicity as determined by MTT reduction assay and morphological changes. To further demonstrate the involvement of GSH and GST in protecting against acrolein and 4-HNE-induced toxicity, buthionine sulfoximine (BSO) and sulfasalazine were used to inhibit cellular GSH biosynthesis and GST activity, respectively. Either depletion of cellular GSH by BSO or inhibition of cellular GST by sulfasalazine led to a marked potentiation of acrolein or 4-HNE-induced toxicity in A10 cells. Taken together, our results demonstrate that both GSH and GST in aortic smooth muscle cells can be induced by DST, and that this increased cellular defense affords protection against reactive aldehyde-induced cardiovascular cell injury.

1405 THE FORMATION OF PEROXYNITRITE AND INDUCTION OF THE INFLAMMATORY MEDIATOR, CYCLOOXYGENASE-2, ARE IMPORTANT FACTORS IN ARSENIC-RELATED CARDIOVASCULAR DISEASE.

M. Bunderson and H. D. Beal. Department of Pharmaceutical Sciences, University of Montana, Missoula, MT.

There is substantial epidemiological evidence linking arsenic-contaminated drinking water with an increased risk of cardiovascular disease. This includes decreased cardiac function as well as peripheral vascular diseases and atherosclerosis. The
1408 THE CORRECTION OF QT INTERVAL IN CONSCIOUS AND ANAESTHETISED BEAGLE DOGS USING DIFFERENT FORMULAE.
S. W. Dean, G. Mestit, F. Mattei and J. Brightwell. Research Toxicology Centre, Pomezia, Italy.
During the last decade some non-cardiovascular drugs already on the market were revised for clinical use on the basis of possible prolongation of QT interval, a proarythmic event which can degenerate into a fatal ventricular arrhythmia (torsade de pointes). Therefore, starting from 1997, QT assessment for human medicinal products has attracted the attention of regulatory authorities. During in vivo preclinical studies, QT interval could be assessed directly from recorded electrocardiograms in conscious or anaesthetised dogs. Nevertheless, the correction factors for heart rate are critical for an accurate and well understood QT interval assessment and the literature suggests that the best formulae to correct QT in dog are those with a minimal correlation with the heart rate. Our laboratory introduced the correction of QT interval as a standard method in pre-clinical dog studies using several different formulae for correction. The electrocardiograms were obtained from 142 (71 males and 71 females) untreated conscious dogs, 24 - 30 weeks old, held in rigid restraint. The mean QT intervals were 178 ms in males and 171.5 ms in females with a range from 140 to 209 ms. The heart rates of both sexes were recorded before (Qp) and after (Qn) the betaxolol oral dose from 287 to 820 ms. QTC were calculated using the Bazett’s square root formula, the Fridericia’s cubic root formula and the Kovacs’ inverted root formula. Results from these studies to address a potential for QT interval prolongation contributed to the design and interpretation of early clinical investigations. The investigation of the potential of pharmaceuticals to prolong the QT interval should be an integral part of pre-clinical safety assessment studies.

1407 METALLOTHIONEIN PROTECTION AGAINST CADMIUM INDUCED CARDIOTOXICITY IN MICE.
L. Elefterie and Y. J. Kang. Pharmacology and Toxicology, University of Louisville, Louisville, KY.
Cadmium (Cd) is a heavy metal environmental pollutant linked to the development of cardiovascular diseases. In murine models of Cd exposure, the effects of Cd are manifested by significant increase in blood pressure as well as heart morphological alterations. The sensitivity of the heart to Cd could be due to the low level of a thick-rich, metal-binding protein, metallothionein (MT), in the tissue. This study was designed to test the hypothesis that elevation of MT in the heart makes this organ resistant to Cd toxicity. A cardiac-specific MT-overexpressing transgenic (MT-TG) mouse model was used and cardiac toxicity was analyzed by evaluating performance of left ventricle and heart morphological changes. MT-TG mice and non-TG controls were treated with CdCl2 (6 mg/kg) for 48 hrs. This treatment lead to a significant increase in heart rate, maximum left ventricle pressure, and the first derivative of intraventricular pressure (dP/dt). On the other hand, the duration of contraction and relaxation and the time constant (Tau) were prolonged. These changes were more pronounced in non-TG than in MT-TG animals. In addition, an increase in contractility caused by isoproterenol (iso) administration in Cd-treated non-TG animals was more pronounced in comparison to saline treated animals whereas, Cd-treated MT-TG animals responded in a similar manner to control. The direct effect of MT on Cd toxicity in cardiomyocytes, the contractile units of the heart was determined using primary neonatal mouse cardiomyocyte cultures. An MT assay was performed for the assessment of cell viability; treatment with 0.1 μM Cd for 1 hr lead to a 65% decrease in non-TG cells whereas this dose lead to only a 50% decrease in viability in MT-TG cardiomyocytes. Results of this study demonstrate that elevation of MT in transgenic animals protects against Cd-induced cardiotoxicity manifested by functional alterations; furthermore, contractility most likely occurred in cardiomyocytes as demonstrated by the results obtained from cell cultures.

1409 A COMPARATIVE HISTOPATHOLOGIC AND METABONOMIC EVALUATION OF VASCULITIS INDUCED BY SKRF 95654, A PHOSPHODIESTERASE III INHIBITOR, IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE WISTAR-KYOTO (WKY) RATS.
J. Zhang, E. H. Herman1, D. G. Robertson1, A. Knapp1, D. Chadwick1 and F. D. Stupple. Center for Drug Evaluation and Research, FDA, Laurel, MD and 1Athen Arbor Laboratories, Pfizer, Inc., Athen Arbor, MI.
The present study was initiated to compare the vascular pathology and metabolic changes induced by SKRF 95654 (SKF) between SHR and WKY. Adult male rats were euthanized 24 h after a single s.c. injection of 100 or 200 mg/kg SKF. SKF caused small vessel vasculitis with arteriolar hemorrhage and necrosis of the mesentery, renal (tubular hyaline droplets, protein casts, glomerular vacuolization), cardiac (necrosis, inflammation, edema), and hepatic (degeneration, inflammation, lesions). Lesions were assessed semiquantitatively. A comparison of the vascular necrosis, inflammation, edema and mean scores of vascular lesions were higher in SHR (4.4 and 5.0) whereas 200 or 100 mg/kg, respectively) than in WKY (0.7 and 0.8 at the same doses). Following dosing with SKF both male cell prevalence and the percentage of mast cells undergoing degeneration in sections of mesentery increased significantly in SHR than in WKY. On the other hand, renal lesions comprised 2.3 were only seen in WKY treated with either dose of SKF. Renal pathology correlated with serum elevations in BUN, creatinine, and GGT seen only in WKY. In both strains significant cardiac and hepatic lesions were seen. Cardiac lesions scores of 4-5 were seen in SHR given 200 mg/kg (10/10) or 100 mg/kg (4/6) compared to (2/6) WKY given the high dose of SKF. Hepatic lesions scores of 2/2 were more frequent in WKY, but without changes in serum ALT, AST, or bilirubin. Metabonomic analyses of urine collected by cisternosentesis revealed alterations in NMR spectral patterns that segregated by treatment and by the sharply different pathologies produced in the two strains. These results indicate that the differences in SKF-induced toxicities between SHR and WKY are providing mechanistic insight as well as strategies for identifying and evaluating biomarkers associated with drug-induced vasculitis.

1410 POLYCYCLIC AROMATIC HYDROCARBONS WITH BAY-LIKE STRUCTURES INHIBITED GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN NEONATAL RAT CARDIOMYOCYTES AND CAUSED ASYNCHRONOUS BEATING.
B. L. Upham1, J. M. David2, J. E. Trosko3 and K. A. Schwartz. 1Pediatrics & Human Development and National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI and 2Human Medicine, Michigan State University, East Lansing, MI and 3Eli Lilly.
Cigarette smoke has been implicated in causing or contributing to many human pathologies including cancer and cardiovascular disease. Polycyclic aromatic hydrocarbons (PAHs) are one class of compounds in this complex mixture of combusted...
by-products of tobacco that has been extensively studied. Although the most abundant PAHs in cigarette smoke are methylated antranecles and phenanthrenes, the toxicity of these compounds has not been extensively studied. Recently, several published reports showed that methyl- or chloro-antranecles, which possess a bay-like structure, induces a release of arachidonic acid, inhibiting gap junctional intercel- lar communication (GJIC), and activates mitogen activated protein kinases in a pleuripotent rat liver epithelial stem cell line. Antranecles with no bay-like structures were inactive. These biological effects are all molecular events associated with the promononal phases of cancer. In the present study, we demonstrated that antranecles with bay-like structures inhibited GJIC in primary cultures of cardiac myocytes from neonatal rats, whereas the antranecle lacking a bay-like structure had no effect on GJIC. This inhibition of GJIC directly correlated with the induction of asynchronous beating, illustrating the importance of GJIC in the metabolic coordination of synchronous contraction of cardiac myocytes. These results indicate that a prominent component of cigarette smoke, namely methylated antranecles with distinct structural configurations, could be a potential etiological agent contribut- ing to cardiac dysfunction.

1411 VASCULAR SMOOTH MUSCLE CELL PROLIFERATION FOLLOWING CHRONIC, INTERRMITTENT EXPOSURE TO 4, 4'-METHYLENEDIANILINE.

T. R. Dugas, B. C. Jones, R. C. Milfin, V. Santa Cruz, P. J. Bort, and M. K. Kang. Dept. of Pharmacology, LSU Health Sciences Center, Shreveport, LA, and Dept. of Pathology, University of Texas Medical Branch, Galveston, TX.

Since 1900, more Americans die annually from cardiovascular diseases (CVD) than from any other disease, and since 1984, more women die from CVD than men. Ironically, few environmental toxicants have been evaluated for their capacities to mediate these diseases. We recently discovered that in female rats weekly, low-dose exposure to 4, 4'-methyleneedianiline (DAPM), a compound used in the produc- tion of polyurethane, results in vascular medial hyalplasia in both the liver and lung. In addition, treatment of vascular smooth muscle cells (VSMC) in culture with micromolar amounts of DAPM results in VSMC proliferation. Relevant to this newly revealed toxicity, Kautaen et al. (1998) have suggested that the majority of DAPM metabolite bound to protein in the liver is formed through metabo- lism by extrahepatic peroxidase enzymes. Peroxidase enzymes metabolize aromatic amines like DAPM to their corresponding imines, which are reactive intermediates capable of binding to DNA and protein. To evaluate whether peroxidase enzymes in VSMC are involved in DAPM-induced VSMC proliferation, we treated VSMC in culture with 0-50 μM DAPM and analyzed the cells for peroxidase activity and the presence of cycloxygenase-2 (COX-2) protein. Western analysis revealed that VSMC express COX-2, and DAPM treatment increased peroxidase activity 12h after exposure. DAPM or its metabolites thus appear to upregulate the expression of COX-2. Finally, VSMC proliferation was attenuated when cells were simultaneously treated with N-acetylcysteine, a non-protein thiol capable of reacting with electrophilic intermediates, thus competing for their reaction with endogenous molecules and possibly preventing cell damage. The toxicity of the data presented here suggest that VSMC themselves are capable of metabolizing DAPM to reactive intermediates. (Supported by F32 ES 05926-01.)

1412 TIME COURSE OF PYRIDOSTIGMINE BROMIDE TREATMENT ON BLOOD PRESSURE AND ACETYLCHOLINESTERASE ACTIVITY IN MICE.

L. Bernatova, E. A. Price, R. D. Grubbs, and M. Morris, Pharmacology and Toxicology, Wright State University School of Medicine, Dayton, OH, and Cardiovascular Physiology, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia.

Pyridostigmine bromide (PB) is used primarily for treatment of myasthenia gravis and for prophylactic protection against organophosphorous nerve poisoning. PB acts by reversible inhibition of acetylcholinesterase (AChE) which is involved in the metabolism of ACh and thus in regulation of neuromuscular and autonomic function. However, there is a little information on the effect of PB on cardiovascular (CV) function. Experiments were performed to determine the time course of the effect of PB on plasma AChE activity and blood pressure (BP) and heart rate (HR) in conscious mice. The experiment used C57 male mice with chronic carotid arterial catheters for CV measurements and collection of blood samples. PB was administered sc using osmotic minipumps at doses of 1 and 3 mg/kg/day for 7 days. The controls were sham-operated with minipumps filled with saline. BP and HR (sampling rate 85 Hz) were measured for 24 hr on day 0 and days 3 and 7 after minipump inser- tion. Sequential blood samples (25 μl) for AChE determination were collected on the same days. Mean BP of the control group was 108±5 and 103±4 mm Hg during the dark and light period, respectively. HR was 499±62 and 478±63 beats/min during the dark and light period, respectively. PB treatment had no effect on BP and HR. Basal AChE activity was 0.44±0.18 umol/min/ml. PB treatment (1 mg/kg/day) had no influence on AChE activity. However, 3 mg/kg/day of PB decreased significantly AChE activity on day 7 by 84±16%. In conclusion, chronic low dose PB exposure in mice decreased AChE activity but had no effect on BP and HR. Supported by DoD contract N00014-95-6.288

1413 TELEMETRY MONITORING OF CARDIOVASCULAR FUNCTION IN CONSCIOUS MALE CYNOGMUS MONKEYS AFTER ORAL TERFENADINE OR ASTEMIZOLE.


A primate telemetry system was developed for its utility in detecting QT prolongation, cardiac arrhythmias or other physiological changes after terfenadine or astemizole. Data collection and analysis was done with an Open-A.R.T./Pomona (Data Sciences International/Gould Instruments) system. A telemetry transmitter designed to monitor blood pressure (BP), core temperature (Temp) and ECG was surgically implanted in each of three male cynomolgus monkeys. The animals were allowed four weeks recovery. Treatments were administered orally (5 mg/kg) at one-week intervals. The dosing sequence was: Control vehicle (0.06% methylcellulose), three doses of terfenadine (30, 100 and 300 mg/kg body weight), control vehicle, three doses of astemizole (10, 30 and 100 mg/kg body weight) and a final control vehicle. Continuous data recording began 2 hours before dosing (0700) and ended 24 hours after dosing (0900 the next day). Animals were recorded on videotape over the same time period (infrared light at night) for subsequent behavior analysis. All parameters were evaluated at half hour intervals before dosing and hourly after dosing. The data files were analyzed for BP heart rate, QT interval and Temp. QTC (Bazett's) increased after treatment in one of three terfenadine and all three astemizole animals. The QT prolongation was readily detected with the hourly sampling protocol. Five fluoride de points (TDP) polymorphic ventricular arrhythmias episodes were detected between 3 and 6 hours after the high astemizole dose in one animal. TDP events were of very short duration (2-4 second) and required continuous monitoring for detection. No other treatment related changes were seen. Heart rate and Temp exhibited consistent diurnal variation during all treatments. In conclusion, the telemetry system and protocol used in this study successfully detected QT prolongation plus TDP after astemizole and QT prolongation after terfenadine. Continuous recording was needed to insure detection of intermittent polymorphic ventricular arrhythmias.

1414 PREVENTION OF DIABETIC CARDIOMYOPATHY BY METALLOTHIONEIN THROUGH SUPPRESSION OF HYPERGLEMIA-INDUCED OXIDATIVE STRESS AND CELL DEATH.

L. Cai, W. Li, X. Sun, Y. Li and Y. J. Kang. Medicine, University of Louisville, Louisville, KY.

Diabetic cardiomyopathy is related directly to hyperglycemia; however, proper treatment strategies are unavailable since its cellular and molecular mechanism is unclear. The present study was undertaken to test the hypothesis that hyperglycemia-induced myocardial injury is mediated by oxidative stress and metallothionein (MT). A potent antioxidant, diacylcardiac cardiomyopathy. A cardiac-specific, MT-overexpressing, transgenic mouse model (MT-TG) and non-transgenic controls (WT) were treated with streptozotocin (STZ). About 70% of the mice treated with STZ-developed diabetes as determined by whole-blood glucose levels. There were no differences in hyperglycemia, body-weight gain, or serum lipid peroxide levels between MT-TG and WT diabetic mice. However, serum creatine phosphokinase activity, a biomarker of myocardial injury, was markedly increased in WT diabetic mice. Correspondingly, myocardial patholog- ical changes as examined by light and electron microscopy and functional alterations as determined by a heart performance analysis were found in WT diabetic mice. Importantly, these diabetic myocardial injuries were all suppressed in MT-TG diabetic mice. In addition, high levels of glucose caused suppression of primary cultures of cardiac myocytes, likely through inhibition of cardiac cell death caused by hyperglycemia-derived oxidative stress. (Supported by NIH, AHA and U of L School of Medicine)
DIFFERENTIAL EFFECTS OF LOW AND HIGH CONCENTRATIONS OF QUINIDINE TO INDUCE TORSADE DE POINTES ARRHYTHMIAS: ROLE OF LATE SODIUM AND DELAYED RECTIFIER CURRENTS.


It is well-known that Torsade de Pointes arrhythmias develop with low, but not high, plasma levels of quinidine. Previous studies have suggested that this may be due to the ability of the drug to amplify transmembrane dispersion of repolarization (TDR) and thus create a vulnerable window at low concentrations of the drug. Low levels of quinidine were shown to dramatically increase TDR, secondary to a preferential prolongation of the action potential duration (APD) of the mid myocardial (M) cell. Selective block of rapid delayed rectifier current (I_k) was shown to mediate this effect.

This study is designed to elucidate the ionic basis for the differential arrhythmogenic effects of low and high concentrations of quinidine. M cells have a smaller slow delayed rectifier current (I_k) but a larger late sodium current (I_Na) compared to epicardial or endocardial cells. We hypothesized that inhibition of I_k and late I_Na underlies the uniform prolongation of APD and the reduction in TDR caused by higher concentrations of quinidine. I_k was measured using a whole cell patch clamp technique and defined as the change in the membrane 3988-sensitive tail current elicited by repolarization to 0 mV after a 3 sec prepulse to 40 mV. M quinidine did not affect I_k (1.57 ± 3.7% inhibition), but 25 microM significantly inhibited the current (79.4 ± 11% inhibition at 25 microM). Late I_Na (the sustained TTT current at the end of a 300 msec depolarizing pulse to 0 mV from -130 mV) was measured using the perforated patch technique. Quinidine (10 and 25 microM) significantly inhibited late I_Na, whereas lower concentrations were devoid of any effect. In addition, to the selective I_k block observed with low concentrations of quinidine, high levels inhibit I_k and late I_Na. Simultaneous block of all three currents is likely responsible for the homogeneous prolongation of transmural APD and lower arrhythmogenicity of higher levels of the drug.

DOXORUBICIN CARDIOTOXICITY IN SPONTANEOUSLY HYPERTENSIVE RATS.


Doxorubicin (DOX) is an anthracycline used in the treatment of cancer. Current use of DOX has resulted in well documented dose-limiting cardiotoxicity. Using spontaneously hypertensive rats (SHR), we investigated gene expression changes associated with DOX cardiotoxicity in heart, liver and blood. DOX was administered to rats by intravenous injection for up to 2 weeks at a dosage of 1 mg/kg/day, resulting in a cumulative dosage of 1,3 to 6 mg/kg. Toxicities were evaluated by a clinical observation, clinical pathology, light microscopy, and ultrstructural pathology.

Dose dependent increase in cTNT levels indicated DOX-induced cardiomyopathy in all treatment groups. This was later confirmed by small multifocal lesions of necrosis observed in all treated groups. Overall 10 genes were regulated in heart, 20 in liver and over 90 in blood. With the exception of a few genes there were few points of overlap in gene expression between treatment groups within a given tissue or between tissues. 12 lipoxigenase was one of these genes and was increased 17 fold in heart, 5 fold in liver and 3 fold in blood in high dose rats. 12 lipoxigenase has been previously implicated for its function in cardiac repair processes and may serve as a marker for DOX-induced cardiotoxicity. Many other genes of interest were regulated in blood after DOX treatment including catalase, transmembrane receptor, BCLs, LEMAC-1 and others. Further studies are ongoing investigating whether functional correlates ascribed to these transcriptional events correlate with cTNT levels and histopathological changes observed in DOX treated rats. In addition, these ongoing studies would help confirm these gene changes and determine their pharmacological or toxicological relevance.

RECRUITMENT OF NUCLEAR RECEPTOR COREGULATORS BY THE PEROXISOMAL PROLIFERATOR ACTIVATED RECEPTOR α.

E. S. Tien and J. B. Yanden. "Veterinary Science, Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA.

The peroxisome proliferator activated receptor α (PPARα) is a member of the nuclear receptor family and regulates genes that are involved in the metabolism of fatty acids in response to peroxisome proliferators (PPs). Coregulators are a class of proteins whose main function is to enhance or repress the transcription of genes that are regulated by receptors such as PPARα.

In these studies, we examined the nuclear protein phosphoP, the RNA coregulator SRA and the PPAR binding protein (PBP). SRA and phosphoP were both able to enhance transcriptional activity of PPARα and can be classified as coactivators of PPARα. PBP is a known coactivator for PPARα, leading to enhanced activation of a PP reporter gene. Activation of PPARα by conjugated alipholic acid (CLA) in the presence of PBP leads to enhanced activation of a PP responsive gene to a greater extent than Wy-14,643. This suggests greater recruitment of PBP to the transcription complex upon activation with CLA. The possibility that coregulator recruitment to nuclear receptor transcription complexes may be ligand specific is being examined.

SELECTIVE DRINKING FOR ALCOHOL PREFERENCE IS PRESENTED ON ACETALDEHYDE DEHYDROGENASE 2 (ALDH2) GENE TARGETING MICE.

T. Ise, K. Kitagawa, A. Matsumoto, T. Oyama, A. Yoshida, K. Nakayama and K. Nakayama. "Environmental Health, University of Occupational and Environmental Health, Kitakyushu, Japan. First Department of Biochemistry, School of Medicine, Hamamatsu Medical University, Shizuoka, Japan. Beckman Research Institute of the City of Hope, Duarte, CA and "Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Fukuoka, Japan.

Aldehyde dehydrogenase 2 (ALDH2) plays a major role in the detoxification of aldehydes. A diminished enzyme activity due to mutation of the ALDH2 gene is associated with high alcohol sensitivity and a low tolerance in humans. The genetic background distinguishing an alcohol preference and avoidance of various inbred mouse strains is not clear. There is no animal model for human ALDH2 alleles. We generated Aldh2 mutant C57BL/6 mice by the targeting knockout, and Aldh2 activity was null in the mitochondrial fractions of the liver of the Aldh2-/-mice (K. Kitagawa et al. 2000). The mice showed selective drinking for alcohol as if a human ALDH2 genotype. The Aldh2-/- mice consumed only one-third the volume of ethanol solution compared to wild-type Aldh2+/- mice. No significant difference were observed in the body weight and food intake between the two strains during the experiment. The Aldh2+/- and Aldh2-/- C57BL/6 mice own same genetic background except Aldh2. Therefore, the present study demonstrates a clear effect of Aldh2 genotype on alcohol preference.

IN VIVO QUANTIFICATION OF SEX-SPECIFIC HEME OXYGENASE-LUCIFERASE (HO-LUC) TRANSGENE EXPRESSION FOLLOWING CHLOROFORM-INDUCED TOXICITY IN MICE.


Heme oxygenase-1 (HO-1) is a key enzyme in the conversion of heme to bilirubin and may be involved in some cellular anti-oxidative responses. The HO gene has been previously shown to be up-regulated very quickly after treatment with chemicals or other inducers of oxidative stress. FVB mice transgenic for the luciferase gene under transcriptional control of the HO promoter (HO-luc) have been shown to express the HO-luc transgene similarly to the endogenous HO gene following the administration of HO-inducing compounds, in vivo tissue-specific expression of HO-luc can be visualized using a low-light imaging system (IVIS)™ after i.p. injection of luciferin. The HO-luc mouse has previously been shown to express luciferase in response to cadmium chloride, a potent HO inducer. In this study, the sex-specific differences in HO-luc expression following chloroform treatment were explored. Male and female HO-luc mice were treated with 100, 200 and 250 mg/kg chloroform either orally or s.c. Male mice showed renal expression of HO-luc whereas female mice did not. Female mice that had been pretreated with testosterone for 3 days prior to chloroform treatment showed HO-luc expression similar to males. This expression pattern was reversed in the same female mice dosed with chloroform 7 days after testosterone depletion. Pathology confirmed the tissue-specific toxicity of chloroform. These findings suggest an androgen-mediated pathway involved in sex-specific chloroform toxicity.

EXPOSURE TO LEAD INCREASES THE ACTIVITY OF SP1-DRIVEN SV40 PROMOTER IN TRANSFECTED PC12 CELLS IN A CONCENTRATION-DEPENDENT MANNER.

V. Balasubramaniam, W. Wei and N. H. Zawis. "Biomedical Sciences, URI, Kingston, RI.

Transcription factors organize and orchestrate differential gene expression, a phenomenon that allows cells to obtain different recipes from the same inherent genome. Zinc finger proteins (ZFP), a class of transcription factors; require Zn for their structural and functional integrity. Hence, ZFPs are susceptible to coexisting heavy metals such as lead (Pb). Sp1, a member of the ZFP family, has been shown to be a cellular target and mediator for Pb-induced anomalies in the regulation of gene expression. Sp1 is a component of a transcriptional complex that is involved in..."
the regulation of a variety of genes responsible for differentiation and development. It has been shown that Pβ mediates Sp1 DNA-binding both in vivo and in vitro. In order to understand the mechanisms involved in Pβ-mediated interactions at the transcriptional level, we transacted PCL2 cells with the vector pGL3 (Promega), which contains a SV40 promoter recognized by Sp1, and a luciferase reporter gene. Twenty-four hours post-transfection, cells were exposed to four concentrations of Pβ (0.01, 0.1, 1 and 10 μM) and nerve growth factor (NGF, 50ng/mL). Luciferase activity was measured at 48 hour, post-exposure. NGF increased the activity of the SV40 promoter consistent with its ability to elevate Sp1 DNA-binding as evidenced by the EMSA studies. Very similar to the growth signal NGF, Pβ exposure resulted in a consistent increase in SV40 promoter activity that was observed in a concentration-dependent manner. Pβ (0.01μM to 10μM) and nerve growth factor (NGF, 50 ng/mL) increased luciferase activity when compared to 1μM and 10μM, which resulted in up to a two-fold increase. To confirm the involvement of Sp1 in the activation of the SV40 promoter, we blocked the Sp1 mRNA by co-transfecting with Sp1 antisense oligonucleotides. The activity of SV40 was reduced following such treatment. These data suggest that both NGF and Pβ can modulate the function of Sp1-driven transcriptional constructs and gene expression may be perturbed during development through interference with the transcription factor Sp1.

1421 DIFFERENTIAL EFFECTS OF 17ß-ESTRADIOL ON VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR EXPRESSION IN ENDOMETRIAL AND BREAST CANCER CELLS LINES.

K. Higgins, M. Stoner and S. H. Safe, Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.

Vascular endothelial growth factor receptor (VEGFR) plays a key role in VEGF-induced angiogenesis. This study investigated the effects of E2 on transcriptional activation of reporter gene (luciferase) activity in HECA1 cells transfected with the following constructs containing VEGFR2 gene promoter inserts - 716 to +268 (pVEGFR-A), -225 to +268 (pVEGFR-B) and -35 to +268 (pVEGFR-C). In HECA1 cells cotransfected with pVEGFR-A and 250 ng of human estrogen receptor α (hERα) expression plasmid, E2 decreased luciferase activity by 50%, and similar results were observed after transfection with pVEGFR-B and pVEGFR-C. E2-mediated downregulation was also observed in MCF-7 human breast cancer cells, but not in ZR-75 breast cancer cells. Further, E2-mediated downregulation of the VEGFR2 gene promoter indicates a region from -60 to -37 that is important for E2-mediated downregulation in MCF-7 cells and E2-mediated upregulation in ZR-75 cells. This region contains two Sp1 sites and mutation of either site alone or both sites results in the loss of the E2-mediated responses in both cell types. E2-mediated activation and downregulation of reporter gene activity in ZR-75 and HECA1/MCF-7 cells, respectively, was reverted after cotreatment with the antiestrogen ICI 182, 780. When HECA1 and MCF-7 cells were cotransfected with pVEGFR-C and HE11, a DNA binding domain (DBD) deletion mutant of ERα, E2-dependent downregulation of this promoter was not observed. This indicates that E2-mediated downregulation of pVEGFR-C in HECA1 and MCF-7 cells is dependent on the ERα DBD. In contrast, HE11 was functional for hormone-mediated induction in ZR-75 cells. Current studies are focused on identifying cis-acting promoter elements and trans-acting factors required for cell-specific modulation of VEGFR2 expression. (Supported by NIH CA76636 and ES09106)

1422 MECHANISM OF HORMONAL REGULATION OF cad GENE EXPRESSION IN BREAST CANCER CELLS.

S. Khan, M. Wormke and S. H. Safe, Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.

The multifunctional cad gene (carboxylate phosphate synthase, arachnoid, dihydrolase) regulates pyrimidine nucleotide biosynthesis and is induced by 17ß-estradiol (E2) and insulin-like growth factor 1 in estrogen receptor (ER)-positive MCF-7 and ZR-75 breast cancer cells. E2 induced reporter gene activity in MCF-7 and ZR-75 cells transfected with pCAD1 which contains a -90 to +115 cad gene promoter insert. This region of the promoter contains two upstream GC-rich motifs that bind Sp proteins and two downstream E-boxes that are important targets in some cells for binding mitogen-induced c-my. In MCF-7 cells transfected with pCAD1, E2 induced reporter gene activity in cells cotransfected with wild-type ERα, ER-11C (DNA binding domain deletion) and ER-TAF1 (D538N, ES42Q and D545N mutations that inactive activation function 2) but not ERβ or ERα mutants that delete N-terminal or C-terminal activation functions 1 or 2, respectively. Moreover, in MCF-7 cells transfected with pCAD1 and a construct expressing a dominant negative form of Sp1 protein, there was complete inhibition of E2-induced transcriptional activation. Deletion analysis of the cad gene promoter showed that only the GC-rich and not the E-boxes were required for E2-responsiveness in MCF-7 cells. In contrast, similar studies in ZR-75 cells demonstrated that both GC-rich and E-box motifs were independently ERβ-responsive indicating significant cell-context dependent differences in hormonal regulation of cad even among ER-positive breast cancer cells. The indirect antigens 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin blocks hormone activation of cad in both cell lines and the mechanism of this response is being investigated. (Supported by NIH CA64081, ES01476 and ES09106)

1423 MECHANISM OF ESTROGEN RECEPTOR α/Sp1-MEDIATED ACTIVATION OF ESTROGEN-RESPONSIVE GC-RICH PROMOTERS.

K. Kim, N. Thu, B. Saville, I. Samudio and S. H. Safe, Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX and Biochemistry & Biophysics, Texas A&M University, College Station, TX.

Estrogen receptor α (ERα/Sp1) activates GC-rich gene promoters in MCF-7 or MDA-MB-231 breast cancer cells, and this response is dependent on activation function 1 (AF1) of ERα. This study investigates contributions of the DNA binding domain (DBD) and AF2 and also promoter context on ERα/Sp1 action in breast cancer cells. In transient transfection studies, wild-type human (hERα) and mouse ERα activated GC-rich constructs containing three tandem Sp1 binding sites (pSp1), or estrogen-responsive GC-rich promoter inserts from the adenomatous polyposis coli (pAPC) or retinoic acid receptor α (pRARα1) genes. The pattern of activation was similar for all three promoters in MCF-7 and MDA-MB-231 cells, and binding of hERα and Sp1 to the endogenous adenomatous polyposis and retinoic acid receptor α gene promoters was observed in chromatin immunoprecipitation assays. The relative contributions of the DBD and AF2 regions of ERα on ERα/Sp1-mediated transcription were determined using human and mouse ERα constructs containing critical deletions (c-fingers 1 or 2) and mutations (AF2:D538A, E542A and D545A). The results indicate that the DBD of hERα was not required for estrogen-dependent activation of hERα but was essential for estrogen-induced activation. Results obtained with AF2 mutants and competition with nuclear receptor box (LSXXLL) peptides from GRIP1 indicate that AF2 of ERα was not essential for ERα/Sp1 action and prototypical AF2-interacting coactivators did not enhance the activity. Thus, the mechanism of ERα/Sp1-mediated gene expression is uniquely AF1-dependent and differs significantly from the classical ERα activation of an estrogen-responsive element promoter or ERα/AF1 modulation of AP1-dependent promoters/gens. (Supported by NIH CA76636 and ES09106)

1424 SARIN CAUSES DIFFERENTIAL ALTERATION OF MRNAS CODING FOR GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP), VIMENTIN AND ALPHA TUBULIN IN THE CENTRAL NERVOUS SYSTEM OF RATS.

T. V. Damadoran, A. A. Abdul-Rahman, M. A. Bilsaka, A. A. Meckai and M. B. About-Dounia, Pharmacy and Cancer Biology, Duke University Medical Center, Durham, NC.

We studied the effect of a single (50 µg/kg, i.m.) dose of sarin (5,5-DL) on the expression of the cytoskeletal genes, GFAP, vimentin (astroglial markers), and alpha tubulin (mostly neuronal) in the central nervous system (CNS) of male rats which were sacrificed at different time points, i.e., 1, and 2 hrs, as well as 1, 3 and 7 days post-treatment. Northern data collected indicate differential / spatial / temporal regulation of all three transcript levels. Both GFAP and vimentin were induced at 1 hr in all tissues except for brainstem. Overexpressed transcript levels of GFAP and vimentin remained high in more sensitive regions like cortex/medulla, while other regions like cortex, cerebellum and spinal cord showed more downward trend for either both GFAP and vimentin at 7 days. Observed changes in these transcript levels may indicate injury response and recovery of astroglia. The immediate downregulation levels of the alpha tubulin transcripts ranged from small (12% in cortex) to high (51% in medulla) except the brainstem, where there was no induction until day one. The levels, however, showed time-dependent increase and reached peak values (240%) at 7 days in the brainstem. The cerebellum showed a great degree of fluctuations in alpha tubulin transcripts levels after immediate induction while the cortex showed more or less the same moderately induced levels for the major part of the time course. Immediate induction and variable persistence of these transcripts in sarin-treated CNS suggest that sarin-induced neurotoxicity is in part mediated by the altered expression of cytoskeletal genes. Furthermore, our data indicate that although sarin induces acute toxicity by disrupting the cholinergic system, the development and persistence of long-term neurological deficits may depend on other pathways in the CNS. Supported in part by US Army Medical Research and Materiel Command under contract DAMD 17-98-C-0027.
1425 VITAMIN D-INTERACTING PROTEIN 150 (DRIP 150) AS A COACTIVATOR OF ESTROGEN RECEPTOR α (ERα)- AND ERα/Sp1-MEDIATED TRANSLATION IN ZR-75 CELLS.
J. E. Lee and S. H. Sae, Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.
Vitamin D interacting protein 150 (DRIP 150) is a member of a family of nuclear receptors that interact with a variety of transcription factors and nuclear receptors including estrogen receptor α (ERα). The effects of DRIP 150 as a coactivator of ERα and ERα/Sp1-mediated translation was investigated in ZR-75 breast cancer cells transfected with constructs containing multiple copies of estrogen response elements (ERE) (pERE3) or GC-rich (pSp1) motifs. In transient transfection studies, DRIP 150 induced a maximal 10- and 4-fold enhancement of estrogen-dependent activation of pERE and pSp1, respectively. The domains of ERα required for DRIP 150-mediated coactivation of ERα and ERα/Sp1 were investigated by cotransfection of inhibitory polypeptides which competitively interact with ERα activation function (AF1) (pAF1) or AF2 (pNR-box). The results showed that ERα/Sp1-mediated transactivation was primarily AF1-dependent, whereas DRIP 150 coactivation involved AF2 of ERα. The following deletion mutants of DRIP 150 have also been generated and these include: DRIP 150m1 (deletion of aa 1145-1454), DRIP 150m2 (deletion of aa 789-1454), and DRIP 150m3 (deletion of aa 325-1454). The results of coactivation of ERα/Sp1 with wild-type and deletion mutants of DRIP 150 indicate that amino acids 789-1145 are required for activity. Current studies are further defining the minimum sequences within DRIP 150 that are necessary for coactivation of ERα/Sp1 and ERα-mediated transactivation. (Supported by NIH CA76636 and ES09106).

1426 PROTEIN INHIBITOR OF STAT-1 (PIAS) AS COACTIVATORS OF ESTROGEN-INDUCED GENE EXPRESSION IN BREAST CANCER CELLS.
W. R. Lee, B. Saville, H. Felix and S. Sae, Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.
The zinc-finger region of the androgen receptor has been used as bait to investigate interacting proteins that may subsequently play a role in modulating gene expression. Research in this laboratory has demonstrated that one of these interacting proteins (SNURF) coactivates estrogen receptor α (ERα) and ERα/Sp1-mediated transactivation in breast cancer cells transfected with constructs containing estrogen response elements (pERE3) and GC-rich (pSp1) promoter inserts. Protein inhibitor of activated STAT-1 (PIAS) has also been identified as an androgen receptor interacting protein (ARIIP) that binds the DNA binding domain, and we have investigated the activity of PIAS proteins as coactivators of ERα and ERα/Sp1 in breast cancer cells. E2 induces reporter gene activity in ZR-75 cells transfected with pERE3 and in cells cotransfected with 5 - 300 ng rat ARIIP (homologous to human PIASα), there was a 3-fold coactivation using 50 ng ARIIP expression plasmid, and significant coactivation was observed using up to 300 ng ARIIP. In contrast, ARIIP coactivated ERα/Sp1-mediated transactivation only 1.7-fold in ZR-75 cells over a smaller range of expression plasmid concentration (5 - 50 ng). In parallel studies, the coactivator activities of human PIASα and PIASγ were also investigated in ZR-75 cells transfected with pSp1. The results showed that PIAS was inactive as a coactivator, whereas PIASγ coactivated a 2.4-fold enhancement of ERα/Sp1-mediated transactivation. Current studies are focused on the mechanisms of PIAS proteins as coactivators of ERα and ERα/Sp1 and domain-specific physical and functional interactions of ERα and Sp1 with PIAS proteins. (Supported by NIH CA76636 and ES09106).

1427 TYROSINE AND SERINE-THREONINE KINASE INHIBITORS BLOCK SODIUM ARSENITE-INDUCED DISRUPTION OF THE ACTIN CYTOSKELETON AND FOCAL ADHESIONS.
T. Suramana1, J. M. Murray2, K. Hu1, T. Papayannidou3, N. Nundharanaranpong4, R. Sridharpulak5, N. Distoin1 and P. Seshasayee1, 2Department of Pharmacology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, 3Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA, 4Department of Biology, University of Pennsylvania, Philadelphia, PA and 5The Institute of Health Research, Chulalongkorn University, Bangkok, Thailand.
Arsenic is a human carcinogen, but the diverse acute physiological effects caused by arsenic are likely due to the activation of various signaling pathways rather than genotoxicity. We used immunofluorescence microscopy to study the effects of 0.25-25 micromolar sodium arsenite on the actin cytoskeleton, focal adhesions, and mitochondrial localization in mouse fibroblasts. At 0.25 micromolar, short-term effects of arsenic were minimal, but at higher concentrations we noted loss of F-actin and vinculin and a concomitant change in cell morphology, from flat and well-spread to round and loosely attached. At 25 micromolar, sodium arsenite caused a severe loss of actin and vinculin and cells detached from the substrate. Signaling by tyrosine and serine-threonine phosphorylation play important roles in regulation of the actin cytoskeleton and focal adhesion formation. Co-treatment with the tyrosine kinase inhibitor genistein or the serine-threonine kinase inhibitor staurosporine completely blocked these toxic effects of sodium arsenite. Studies currently underway will identify the specific members of these kinase families that appear to mediate the toxic effects of sodium arsenite.

1428 COCAINE INDUCES A DOSE-DEPENDENT ALTERATION IN THE EXPRESSION IMMEDIATE EARLY GENES C-FOS AND SP-1 AND IN NUCLEAR FACTOR NF-κB IN PC12 CELLS.
Cocaine is a widely used drug of abuse and psychostimulant which acts on the central nervous system by blocking the dopamine re-uptake sites. PC12 cells, a rat pheochromocytoma clonal line, which in the presence of nerve growth factor (NGF), multiply and differentiate into competent neurons that can synthesize, store and secrete the neurotransmitter dopamine (DA). In the present study, we evaluated the effect of increasing doses of cocaine on the expression of immediate early genes (IEG), c-fos and Sp-1 and a closely related nuclear factor, NF-κB 24 h after the exposure to cocaine (50, 100, 250, 500, 1000, 2500 μM) in NGF-differentiated PC12 cells. Cocaine (50-500 μM) resulted in significant induction of the expression of c-fos, Sp-1 and NF-κB. However, higher concentrations of cocaine (1000 & 2500 μM) resulted in the down-regulation of these expressions after 24 h. We also evaluated the effect of increasing doses of cocaine on the significant dose-dependent decrease in the concentration of dopamine was observed 24 h after the exposure of PC12 cells to cocaine. Therefore in the present study, we reported that cocaine has both upstream and downstream regulatory actions on some IEGs and nuclear factors which is independent of its action on the dopaminergic system. Further studies are underway to explore more mechanistic details.

1429 INDUCTION OF p53 GENE EXPRESSION IN MCF-7 BREAST CANCER CELLS BY 1β-ESTRADIOL IS MEDIATED BY CALMODULIN KINASE IV-DEPENDENT ACTIVATION OF AN NF-κB/CRET-1 COMPLEX.
C. Qin1, T. Nguyen1, J. Stewart1, I. Samudio1, R. B. Burchardt1 and S. H. Sae1, 1Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX, and 2Veterinary Anatomy & Public Health, Texas A&M University, College Station, TX.
In this study, the authors investigated the role of 1β-estradiol (E2) induced tumor suppressor gene p53 mRNA and protein levels in MCF-7 human breast cancer cells. Analysis of the p53 gene promoter identified a minimal E2-responsive region (-106 to -30) that binds multiple transcription factors, but only motifs that bind C/EBP-1 and NF-κB are required for hormone-induced activation of p53-10 (contains a -106 to -30 p53 gene promoter insert). The p56 subunit of NF-κB was identified in both nuclear and cytosolic fractions of untreated MCF-7 cells, and induction of p53 by E2 was inhibited by the IκB super repressor and precursor-derived inhibitor containing the C-terminal region of p105, the precursor of p50. Although nuclear p65 is required for hormonal activation of p33, formation of nuclear NF-κB complex was essentially E2-independent. Therefore we investigated direct activation of p53 in MCF-7 cells in transient transfection studies using p53-10 and a chimeric protein containing the C-terminal 30 amino acids of p65 (p65-p53C) fused to the yeast G4L protein DNA binding domain. Results of studies using selective kinase inhibitors showed that only KN-93, an inhibitor of calmodulin kinase IV (CaMKIV), blocked hormone activation of p53-10 or pm-p53, and inhibition was also observed with cyclohexyl gynostemma-α-M, a cell permeable chelator of divalent calcium ions. In addition, constitutively active CaMKIV also activated pm-p53C in MCF-7 cells. Our results demonstrate that p53 is upregulated by E2 in MCF-7 cells through direct kinase-dependent activation of p53 and cooperative p53-CTF-1 interactions. (Supported by NIH ES09253 and ES09106).

1430 ANALYSIS OF ESTROGEN RECEPTOR α (ERα) AND Sp1 PROTEIN INTERACTIONS WITH PROMOTERS OF ESTROGEN RESPONSIVE GENES IN BREAST CANCER CELLS BY CHROMATIN IMMUNOPRECIPITATION.
I. Samudio and S. H. Sae, Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.
Studies in this laboratory have identified GC-rich elements in the promoters of the cyp17 D (CD), retinoic acid receptor α (RARα) and adenosine deaminase (ADA) genes that bind estrogen receptor α (ERα)/Sp1 in vitro and are required for
estrogen (E2)-induced transactivation. Interactions of ERα, Sp1 and other nuclear transcription factors with specific E2-responsive regions of the CD, RARα1 and ADA gene promoters were investigated by the chromatin immunoprecipitation (ChIP) assay. Briefly, MCF-7 human breast cancer cells were treated with 10 nM E2 for 30, 60, 90 or 120 min; formaldehyde was then added to crosslink proteins and DNA; isolated nuclei were obtained and sonicated for 4·60 sec to obtain chromatin with appropriate fragment length (500-1000 bp). ERα and Sp1 antibodies were used to immunoprecipitate chromatin associated with these proteins and, after removal of the crosslinks and extensive purification, DNA from immunoprecipitated fractions was analyzed by PCR with primers to detect E2-responsive regions of the CD (−2041+17), RARα1 (−1871+10) and ADA (−1486+51) gene promoters. PCR analysis demonstrated that both ERα and Sp1 proteins occupied the CD gene promoter for at least two hours after treatment with E2 and, in contrast to previous reports, cyclic occupation of the promoter by these proteins was not detected. Using a similar approach, we also observed occupation of the ADA and RARα1 gene promoters for up to 120 min by both ERα and Sp1 proteins; however, there was some variability in ERα-promoter interactions that were dependent on the antibody used in the immunoprecipitation step. Current studies are refining this technique, extending the time-course studies, and determining interactions of other coactivator proteins with these gene promoters. (Supported by NIH CA76636 and ES09106).

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VITAMIN D INTERACTING PROTEIN 205 (DRIP 205) AS A COACTIVATOR OF ESTROGEN RECEPTOR α/Sp1-MEDIATED TRANSCRIPTION.
Q. Wu and S. H. Safe. Biochemistry & Biophysics, Texas A&M University, College Station, TX and Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.

Several independent studies have identified vitamin D interacting protein 205 (DRIP 205) as a member of a family of mediator complex proteins that associate with transcription factors and hormone receptors. DRIP 205 coactivates 17β-estradiol (E2)-induced gene expression in ZR-75 and other breast cancer cell lines transduced with wild-type estrogen receptor α (ERα) and constructs containing estrogen response element (ERE) or GC-rich motifs (Sp1) that bind ERα/Sp1. Ten nM E2 alone induced reporter gene activity in ZR-75 cells transfected with pSp1, and wild-type ERα (HE11) (a DNA binding domain deletion mutant) or HE11-TAP1 (point mutations inactivating activation function 2 (AF2)). Cotransfection with DRIP 205 coactivated wild-type/mutant ERα 3.8-, 2.7- and 1.7-fold, respectively. DRIP 205 contains 1566 amino acids including two nuclear receptor (NR)-boxes containing LXXLL motifs (within the aa 528-714 sequence) that interact with helix 12 of the AF2 domain of ERα. Plasmids expressing aa 1-714 (DRIP 205L), aa 516-1366 (DRIP 205M2), and aa 528-714 (DRIP 205M3) were prepared by PCR. The coactivator activity of mutant DRIP 205 expression plasmids was investigated in ZR-75 cells transfected with pSp1 and ERα. Both N-terminal (DRIP 205M2) and C-terminal (DRIP 205M3) deletion mutants of DRIP 205 coactivated ERα/Sp1-mediated transactivation 3.6- and 2.0-fold, respectively, whereas DRIP 205QNR-mediated transactivation of specific domains of DRIP 205 required for coactivation of ERα/Sp1. (Supported by NIH CA76636 and ES09106).

1434
THE UTILIZATION OF LARGE-SCALE INSERTIONAL MUTAGENESIS (GENE TRAP) SCREENS TO IDENTIFY AND DISRUPT NOVEL GENES INDUCED BY TCDD IN MOUSE EMBRYONIC STEM CELLS.

Polychlorinated dibeno-p-dioxins (PCDDs) are industrial compounds or by-products which have been identified in extracts from diverse environmental matrices including fish, wildlife and humans. PCDDs are complex mixtures of congeners with a limited number being ubiquitous in environmental samples. Recently, PCDDs have been the focus of extensive toxicological studies and serious public debate. As a result of this, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been identified as the most toxic congener in the PCDD family and has been shown to elicit numerous adverse responses in animals including neoplasia, immunosuppression, hepatotoxicity, neurotoxicity, reproductive toxicity, and teratogenesis. While TCDDs physiological effects are well known in animals, the overall risk that TCDD poses to humans is unclear. Moreover, outside of the classical dioxin-inducible gene battery, little is known about the complex molecular actions of TCDD, which are likely to be associated with many observed physiological effects. To address this issue, we have developed an experimental strategy that simultaneously identifies TCDD-inducible genes, mutates them, tags them with a reporter molecule, and enables the production of knockout animals carrying the induced mutations. This process has been termed "gene-trapping." The power of this technology is the ability to discover novel genes responsive to a particular chemical, rather than testing the responsiveness of a known gene. Within the scope of this research, we have conducted the molecular and functional characterization of the role of these genes in knockouts and animals. To date, 26 TCDD-sensitive genes have been identified out of approximately 2000 mutated genes, 12 of those genes have been sequenced. The generation of a knockout mouse for one identified gene is currently in progress.

1435
TRAPPING GENES ASSOCIATED WITH TOBACCO USE.

Data from epidemiological studies have strongly linked the development of squamous cell carcinomas of the upper aerodigestive tract to consumption of tobacco, particularly when combined with alcohol. The exact mechanisms leading to

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bacco use to disease are not well understood, but for tumorigenesis these mechanisms are surely genetic. Understanding how carcinogens induce tumor formation ultimately relies on identifying and characterizing the genes that are targeted for mutation and/or dysregulation by these carcinogens. To this end, many mutations have been found in cancer cells from tobacco users, but not all mutations have been associated with specific genes. Thus, the goal of this project is to identify, mutate and characterize those genes. This goal is part of the long-term objective of understanding the genetic mechanisms underlying the development of cancer. To address this goal we have deployed a gene trapping strategy to identify tobacco-sensitive genes. Briefly, mouse embryonic stem (ES) cells are transfected with a promoterless reporter gene construct. Successful transfection of an ES cell will knockout an endogenous gene, but the new gene will be inserted into the same intron under the control of the endogenous promoter. Large numbers of unique gene-trapped ES cell clones are then screened in the presence or absence of cigarette smoke condensate (CSC), and altered reporter gene expression is identified and recorded. The reporter gene itself is then used as the starting point for sequencing of the endogenous genes. Sequences are then compared to those in the GeneBank database to identify novel genes as well as previously known genes that were unknown to be altered/deregulated by tobacco. In addition, mutant ES cells created as described above can be used to generate knockout mice. Those mice, in turn, will then be studied to understand the role of each gene relative to the development of cancer and disease.

1436 PREGNANE X RECEPTOR MEDIATES THE INDUCTION OF RAT ORGANIC ANION TRANSPORTING POLYPEPTIDE 2 BY FREGNE NONOLONE-16 ALPHA-CARBOXYLATE.

G. L. Guo, J. L. Staudinger and C. D. Klaassen, Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

The rat organic anion transporting polypeptide 2 ( oatp2; Slc21a5) is a hepatic sinusoidal transporter that mediates the uptake of a variety of structurally diverse compounds, and has a very high affinity for cardiac glycosides. Treatment of rats with pregnenolone-16 alpha-carboxylate (PCN), a potent ligand for the rodent pregnane X receptor (PXR), significantly enhances the transcription of the oatp2 gene leading to subsequent increases in hepatic oatp2 mRNA and protein levels. In an effort to understand the molecular mechanism of oatp2 induction by PCN treatment, approximately 8700 bp of the 5' flanking region of the rat oatp2 gene were linked to the luciferase reporter gene and used in transfection assay experiments in H4IIE cells. PCN treatment induced the expression of the reporter gene in a dose-dependent manner. A total of four potential PXR response elements (PPRREs) were identified in the 5' flanking region of the rat oatp2 gene. One element (DR3-1) is located approximately -5000 bp with three more (DR3-2, DR3-3, DR3-4) clustered at about -8000 bp upstream of the predicted transcription start site. Results gathered from Electrophoretic Mobility Shift Assays showed that the PXR-RXRalpha heterodimer directly binds to the DR3 with the highest affinity, followed by the DR3-4 and DR3-1, but only to the DR3-3 weakly or not at all. Furthermore, a series of partial deletions of the 5 flanking region of the rat oatp2 gene illustrated that both the proximal and distal clusters of PPRREs are required for maximal induction of oatp2 by PCN. In conclusion, these data elucidate the molecular mechanism by which PCN treatment induces oatp2 gene expression. In addition, this study identifies rat oatp2 as a direct PXR-targeted gene and further supports the hypothesis that activation of PXR affects a network of genes that is involved in either metabolism or transport of drugs, steroids, and bile acids. (Supported by NIH grants ES-09/94 and ES-07079)

1437 ENDOCRINE REGULATION OF RAT ORGANIC ANION TRANSPORTER 2 (OAT2) mRNA EXPRESSION IN KIDNEY.

S. C. Buist, N. J. Cherrington and C. D. Klaassen, University of Kansas Medical Center, Kansas City, KS.

Initially, rat Oat2 was described as a liver-specific transporter. However, recent data indicate gender divergent expression of Oat2 in which male levels are highest in liver while female levels are highest in kidney. Furthermore, in kidney there was a female-predominant pattern of Oat2 evident after the onset of sexual maturity. Therefore, the goal of this study was to investigate endocrine involvement in the elevation of oat2 mRNA levels in female as compared to male kidney. Sex steroid influence was determined by comparing Oat2 mRNA levels in kidneys of castrated and ovariohysterectomized rats to those of intact control rats. Additionally, endocrine effects were studied in hypophysectomized (HX) rats randomly placed in one of the following four treatment groups: 1) testosterone (daily); 2) estrogen (daily); 3) male-pattern growth hormone (two injections per day to simulate pulsatile secretion); or 4) female-pattern growth hormone (continuous infusion via mini-osmotic pumps). Hormone treatments were administered for 5 days. Oat2 mRNA levels were measured by branched DNA signal amplification. Levels of Oat2 mRNA were 10-fold higher in control female kidneys as compared to control male kidneys. Interestingly, gonadectomy did not affect male or female Oat2 mRNA levels, but HX did decrease oat2 levels in females by 50%, resulting in Oat2 mRNA levels in kidney of HX females equivalent to those in kidneys of control male rats. Neither testosterone nor estrogen administration altered Oat2 mRNA levels in kidneys of HX rats. Like Oat1, Oat2 mRNA levels in kidneys of HX male rats were substantially higher than those in control Oat2 mRNA levels in kidneys of HX males or HX females. However, continuous infusion of rat growth hormone, which mimics female-pattern secretion, did increase renal Oat2 mRNA levels in both HX male and HX female rats. Therefore, these data demonstrate that the gender difference in Oat2 mRNA levels in kidney is not the result of sex steroid influences, but rather a function of female-pattern growth hormone secretion. (Supported by NIH grants ES-09/716 and ES-07079)

1438 TISSUE EXPRESSION OF THE RAT UDP-GLUCURONOSYLTRANSFERASE SUPERFAMILY.

M. K. Sheby, N. C. Cherrington, N. R. Vansel and C. D. Klaassen, University of Kansas Medical Center, Kansas City, KS.

UDP-glucuronosyltransferases (UGTs) are a superfamily of phase II drug metabolizing enzymes that catalyze the conjugation of glucuronic acid to a broad spectrum of endobiotic and xenobiotic substrates. The rat UGTs have been divided into two gene families, UGT1 and UGT2, which exhibit a number of differences in tissue distribution. The complete tissue expression of rat UGTs has not been thoroughly examined, but could provide further indication of individual UGT functions. The aim of this study was to quantitatively determine the mRNA levels of rat UGT1 and UGT2 families in various tissues using the branched DNA signal amplification method. UGT1A1 was present at high levels in all tissues examined except lung. UGT1A2 was detected primarily in small intestine. UGT1A3 was detected primarily in liver at low levels. UGT1A6 was present predominantly in kidney and gastrointestinal (GI) tract, whereas UGT1A7 was detected in the GI tract and at low levels in lung. UGT2A1 was detected at high levels only in nasal epithelium. UGT2B1 was detected only in liver, UGT2B3 and 2B6 were detected mainly in liver, whereas 2B8 was detected in stomach, duodenum, and kidney at low levels. UGT2B12 was primarily localized in liver and kidney, with lower levels in duodenum. Finally, UGT1A3, 1A8, and 2B2 were difficult to detect. In summary, the UGTs present in liver include UGT1A1, which was present at high levels, and 2B1, 2B3, 2B6, 2B12, 1A5, and 1A6, which were present at low levels. In both small and large intestine UGT1A1, 1A6, and 1A7 were present at high levels, but 1A2, 2B8, and 2B12 were present in small intestine at low levels. UGT1A1 and 1A6 were present at high levels in kidney, in which lower levels of 2B12 and 2B8 were detected. UGT1A1, 1A6, 1A7, and 2B12 were present at low levels in lung. Only UGT1A1 was present at high levels in brain, with 1A5, 2B3, and 2B12 at low levels. The tissue specific expression of UGTs may be responsible for the tissue specific detoxification or activation of UGT substrates and thus suggests a possible role in target organ toxicity. (Supported by NIH grants ES-09/716 and ES-07079)

1439 LIPOPOLYSACCHARIDE-MEDIATED REGULATION OF HEPATIC TRANSPORTER mRNA LEVELS IN RATS.

N. J. Cherrington, A. L. Slitt, N. Li and C. D. Klaassen, Pharmacology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

The major function of hepatic transporters is to move a wide range of organic substances across the sinusoidal and canalicular membranes. During extrahepatic cholestasis, some hepatic transporters that are involved in the normal vectorial flow of organic substances from blood to bile, such as Mdr2, Mrp2, Mrp3, and Mrp4, are known to be dramatically down-regulated, while other transporters such as Mrp3 are up-regulated. Unlike models of extrahepatic cholestasis where alterations in transporter expression are in response to the accumulation of bile constituents in the hepatocyte, lipopolysaccharide (LPS) may directly alter transporter expression leading to cholestasis characterized by impaired bile flow. The aim of this study was to quantitatively determine the effect of LPS on the mRNA levels of hepatic transporters in Sprague-Dawley rats and whether dexamethasone pretreatment could modulate the LPS-mediated changes in hepatic transporter mRNA levels. Sixteen h after LPS (4 mg/kg) administration, the branched DNA signal amplification assay revealed that hepatic mRNA levels of Mrp2, Mrp6, Mdr1a, Oppl, Oapt2, Lt, Ntcp, Bsep, Oct1, and Oat3 were dramatically decreased, but Mrp5, Mdr2, and Oat2 levels remained unchanged. In contrast, LPS administration increased mRNA levels of Mrplp1, Mrp3, and Mdr1b. A 1 h pretreatment with dexamethasone (10 mg/kg) significantly diminished the reduction of Mdr1a, Oapt1, Oapt2, Lt, Ntcp, Bsep, Oct1, and Oat3 whereas a 1 h LPS pretreatment failed to decrease Mrp2, Mrp6, Lt, Bsep, and Oat3 levels. Dexamethasone also blocked the LPS-mediated increase in Mrp1, Mrp3, and Mdr1b mRNA. The dexamethasone abatement of both LPS-mediated suppression and induction of transporters indicates...
that these responses may be mediated through similar pathways. Moreover, the prevention of LPS-mediated effects by dexamethasone pretreatment on some, but not all transporters underscores the complex nature of hepatic transporter regulation. (Supported by NIH grants ES-09716, ES-09649, and ES-07079)

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DOWN-REGULATION OF LIVER-SPECIFIC TRANSPORTER (Leu Or Oatp4) BY LIPOPOLYSACCHARIDE THROUGH TOLL-LIKE RECEPTOR 4 (TLR4).

N. Li, S. Choughun, N. J. Cherrington and C. D. Klaassen, Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

Lipopolysaccharide (LPS) is a component in the outer membrane of Gram-negative bacteria. LPS stimulates the immune system and causes a dramatic systemic reaction known as sepsis, which can cause cholestasis in humans and rodents and is associated with the down-regulation of specific transporters (Leu or Oatp4) and other transporters in rat liver. Biological action of LPS is thought to be mediated by toll-like receptor 4 (TLR4). The purpose of this study was to determine (1) whether LPS treatment decreases the levels of Lst mRNA in mice and (2) whether TLR4 plays a role in the LPS-mediated decrease of Lst expression. To address this hypothesis, C3H/HeJ mice were used because they are LPS-hyporesponsive due to a missense mutation (F712H) in TLR4. LPS-responsive C3H/OuJ mice were used as controls.

Both C3H/HeJ and C3H/OuJ mice (25-30 g) were injected with a single, nonlethal dose of 5 mg/kg LPS ip in saline. Livers were excised at various time points; 0, 1.5, 3, 6, 12, 16, 24, and 48 h after treatment, and the levels of Lst mRNA were analyzed by real-time branched DNA signal amplification. In control C3H/OuJ mice, TLR4 treatment decreased Lst mRNA levels rapidly in a time-dependent fashion, reaching the minimal level (about 80% decrease) at 12 h following treatment, and returning to normal levels over the following 36 h. In the TLR4 mutant mice (C3H/HeJ), the decrease in Lst mRNA levels was much less pronounced (about 40% decrease) than that observed in the controls (C3H/OuJ). A complete ablation of the response to LPS was not expected in the TLR4 mutant mice (C3H/HeJ), because these mice are hyporesponsive but not unresponsive to LPS. These data support the hypothesis that the LPS-mediated decrease in the expression of Lst is through TLR4. (Supported by NIH grant ES-09649)

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J. M. Maher, N. J. Cherrington and C. D. Klaassen, Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

Multidrug Resistance Proteins (Mps) are a class of ATP-dependent transporters distinguished by their ability to confer resistance to anti-cancer drugs. Two of the newest family members, Mrp5 and Mrp6, were only recently cloned and have not been well characterized. The purpose of this study was to determine the tissue distribution of Mps5 and 6, and to ascertain whether hepatic mRNA levels of the transporters were altered by cholestasis in 6-hour-old mice. Mps5 mRNA levels were quantified using the branched DNA signal amplification method. Transporter mRNA levels were examined in Sprague-Dawley rats for both Mps5 and Mps6 in various tissues. Additionally, microsomal enzyme inducers representing six different transcriptional activation mechanisms were utilized to determine effects on Mps5 and 6 mRNA levels. In a subsequent study, bile duct ligation was used to model cholestasis, and the transcriptional regulation of Mps5 and 6 was subsequently examined. Mps5 mRNA levels were detected predominantly in the adrenal gland, followed by cerebral cortex, cerebellum, and stomach. Expression of Mps5 in the adrenal gland was substantially higher than that present in other tissues. In contrast, Mps6 tissue expression was more ubiquitous, but expression was highest in intestinal tissue, liver, and kidney. Mps5 mRNA expression was minimal in the liver, and neither treatment with microsomal enzyme inducers nor bile duct ligation altered mRNA levels. Mps6, while present in liver, was shown to alter bile by these treatment conditions. In conclusion, Mps5 is most highly expressed in the adrenal gland, while Mps6 is mainly expressed in extrahepatic organs (liver, intestine, and kidney), suggesting markedly different functions. Additionally, the hepatic mRNA levels of Mps5 or Mps6 are not altered in response to challenges such as bile duct ligation or microsomal enzyme induction. (Supported by NIH grants ES-09716 and ES-07079)

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SIGNAL TRANSDUCTION PATTERNS IN VARIOUS MOUSE IMMUNE SURVEILLANCE CELLS AFTER XENOBIOTIC STIMULATION.


With the exception of stem cells, as a general rule cells are specialized to perform a primary function or limited set of functions unique to its type or subset. Immune cells, as a subset, have functions that are distinguished by the role that particular cell plays in the inflammatory process. A determining factor of this role is based, in part, on the mediators (or endpoints) released from a cell, which regulate the inflammatory response. The activation and release of these endpoints are controlled by complex intracellular signaling mechanisms. A single signaling molecule, such as LPS or PMA, can activate a variety of endpoints (genetic changes occurring at the termination of a signal transduction cascade) in different subsets of immune cells. Multiple signal transduction pathways can be activated to create unique patterns of activation (signature response), which may or may not be toxicant and cell specific. In order to determine the specificity of this response, alveolar macrophages, splenic dendritic cells, and PMNs (variable surveillance cells impacted by the in inflammatory process) were isolated from mice and stimulated with either LPS or PMA. Genomic array analysis of cells stimulated with either LPS or PMA revealed a unique expression profile for signal transduction pathways for each toxicant that was common to all cell types, though endpoints varied considerably among the cell types. These data suggest that intracellular "signature response" occurs which are unique to the inducing toxicant or class of toxicants, but common to all cells or subset of cells. These "signature responses" can be utilized to develop a mechanistic understanding of toxicity and explored to develop rapid, cost effective in vitro assays. Understanding mechanisms of intracellular signal transduction can lead to improved in vitro screening assays which ultimately may reduce animal usage and provide less expensive assays for screening potential toxicants.

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ALTERNATIVE PROCESSING OF THE HUMAN FM06 TRANSCRIPT.

K. A. Hopp and R. N. Hing, Pediatrics, Medical College of Wisconsin, Milwaukee, WI.

The flavin-containing monooxygenases (FMO) are important for the metabolic disposition of a wide variety of environmental toxicants and therapeutics. There is evidence for five distinct mammalian flavin-containing monooxygenases (FMO1-5) each exhibiting a highly regulated tissue, developmental, and species-specific expression pattern. A sixth FMO gene sequence is found within the FMO cluster on the long arm of human chromosome 1. Although this gene exhibits the expected 48-55% sequence identity with FMO1. FMO2, FMO4 and FMO5, it is unusual in that it shares 70% sequence identity with FMO3. Sequence analysis revealed the presence of the conserved NADP+ and FAD binding elements typical of this gene family and predicted normal splicing to yield a 1.649 base plus transcript encoding a 539 amino acid protein. However there has been no evidence of FM06 expression or demonstration of specific enzyme activity. In this study, RNA isolated from human liver and the FM06 cDNA was amplified by nested RT-PCR. Products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Individual amplification products were purified and sequenced using the PCR primers. The expected cDNA amplification products were not observed in any of the samples. Rather, truncated versions were seen in the range of 1, 100 to 1, 400 bases. Examination of the DNA sequence revealed splice variants which either eliminated or shortened some of the expected exons. Comparison to FMO3 revealed sequence changes at each of the alternative splice sites confirming to consensus splice donor or splice acceptor sites. Open reading frame analysis of these variant transcripts indicated a loss of competency to encode full length functional enzyme and suggests that FM06 activity is not present in hepatic tissue and that this gene represents a pseudogene. Further studies will examine FM06 splicing scenarios in other tissues. (Supported in part by PHS Grant No. CA53106).

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p', DICHLOORODIPHENYL DICHLOOROETHYLENE (p', DDE) INHIBITS THE ANDROGEN-INDUCED ANTIPROLIFERATIVE RESPONSE IN CAMA-1 CELLS.

P. Ayotte and C. Larochelle, Public Health Research Unit, CHUQ-CHUL, Laval University, Beaumont, PQ, Canada.

We recently reported that p', DDE, the main metabolite of p', DDT and a potent anti-androgen, is associated with tumor aggressiveness in women diagnosed with breast cancer (Cancer Epidemiol. Biomarkers Prev. 2000; 9: 161-6). Since androgens are known to inhibit the growth of breast cancer cells, we tested the hypothesis that p', DDE might block this antiproliferative response in the estrogen-responsive CAMA-1 human breast cancer cell line that expresses the androgen receptor. CAMA-1 cell proliferation was determined by seeding 2 x 10⁶ cells into 24 well plates and exposing the cells to various concentrations of 17-betadrol (E2), dihydrotestosterone (DHT) or p', DDE for 12 days. Cells were then fixed with methanol and the effect on cell growth was determined by measuring DNA in the cells by fluorometry, following the addition of DABA. The maximum proliferative response was observed with 0.1 nM E2. DHT added alone to the cell medium did not increase cell proliferation, but 0.1 and 1 nM DHT reduced the proliferative response induced by 0.1 nM E2 by 30% and 50%, respectively. p', DDE had no effect by itself on CAMA-1 cell growth. However, in combination with 0.1 nM E2 and 0.1 nM DHT, increasing concentrations of p', DDE in the incubation
MECHANISM OF P53 ELEVATION BY BUTADIENE DIEPOXIDE IN HUMAN FIBROBLASTS

P. M. Muganda, S. Y. Seemanspalli and M. Starks. Biological Sciences, Southern University, Baton Rouge, LA. Sponsor: T. Egan

The environmental chemical butadiene is prevalent in petrochemical industrial areas, and is a known mutagen and suspected human carcinogen. The molecular mechanisms of butadiene toxicity, however, are not yet well understood, though the cellular effects of this compound are mediated through its metabolites. Since the tumor suppressor p53 protein responds to cellular stress, and mediates cellular responses to environmental stress and DNA damage, we investigated whether p53 levels were affected in human embryonic lung fibroblasts exposed to butadiene diepoxide, the most genotoxic metabolite of butadiene. We found that under experimental conditions where glyceraldehyde-3-phosphate dehydrogenase (G3PDH) levels remained unchanged after western blot analysis, butadiene diepoxide was effective in elevating p53 levels in exposed fibroblasts at concentrations as low as 1 microMolar. Levels of p53 were elevated approximately 40 fold as compared to control fibroblasts. Further investigation by pulse chase analysis revealed that p53 stability was mainly responsible for the elevated p53 levels in butadiene diepoxide exposed fibroblasts. Our data also revealed that levels of acetylated p53 at lysine 382 increased in correlation to the elevated p53 levels in butadiene diepoxide exposed cells. Since p53 acetylation has been found to be important in stabilizing and regulating p53 function in response to genotoxic stress in other systems, our results suggest that p53 acetylation at lysine 382 may play a similar role in human fibroblasts exposed to butadiene diepoxide.

TUMOR FORMATION IN MICE BY STYRENE. INDUCTION OF CELL PROLIFERATION IN THE LUNG BY STYRENE METABOLITES


Styrene is a high production volume chemical (approx. 2x10^6 tons/year) used as a monomer for plastic productions. Mice develop lung tumors when exposed chronically to styrene. The relevance of these findings for humans is under investigation. Epidemiological data provide no evidence for a carcinogetic potential in humans. Styrene is metabolised to styreneoxide. This electrophilic metabolite is assumed to cause genotoxicity and consequently lung tumors in mice. Research on the metabolism, kinetics and genotoxic effects of styreneoxide in mice and rats did not show a clear-cut model for tumor formation. The mechanism of styreneoxide cannot be seen as the only ultimate toxicant. Recent mechanistic studies demonstrated that the induction of cell proliferation in the lungs of mice is a major cause of tumor formation by styrene rather than a genotoxic effect. We tested styrene and five of its metabolites (β-phenylethanol, β-phenylethanol, phenylethylacetol, acetophenone, phenylactic acid) for induction of cell proliferation in the lungs of CD-1 mice and Fisher rats. Test substances were administered i.p. The cell proliferation in the lungs was quantified by the β-phase response (BrdU labelling index). It was determined 24 and 72 hours post application in large and medium bronchi, terminal bronchi and alveoli. Highest increase of cell proliferation were observed in the alveoli of mice treated with β-phelyethanol, phenylethylacetol and phenylactic acid, with the aldehydes causing the most pronounced effect. This was observed 24 and 72 hours post application. Interestingly the oxidation at the β-carbon of styrene is an important pathway in mice, but not in rats. This metabolism leads to phenylethylacetol and phenylactic acid, the most potent inducer of cell proliferation predominantly on styrene. The considerations of these metabolites to the tumour formation by styrene exposure would explain the differences between mice and rats and may provide a better basis for the assessment of risks in humans.

FUNCTIONAL CHARACTERIZATION OF C190T POLYMORPHISM IN HUMAN N-ACETYLTRANSFERASE 2 (NAT2) GENE

Y. Zhu, M. A. Doll and D. W. Hein. Pharmacology and Toxicology, University of Louisville, Louisville, KY.

N-acetyltransferase 2 (NAT2) is an important phase II enzyme, which catalyzes N-acetyl (deconjugation) and O-acetylation (activation) of many drugs and environmental carcinogens. Genetic polymorphisms in the NAT2 gene have been associated with differential susceptibility to cancers and drug toxicity from these compounds. Single nucleotide polymorphisms (SNPs) have been identified in the human NAT2 coding region. Their effects on NAT2 expression, stability, and catalytic activity have been investigated in several allele NAT2*19 recently was identified. This variant allele possessing the C190T (R64W) SNP was cloned and expressed in yeast (Schizosaccharomyces pombe). Substantial reduction in expression of NAT2 immunoreactive protein and protein stability were observed for NAT2*19 (half-life-2.71 min) when compared with NAT2*4 (half-life-14.6 min). No significant differences in mRNA expression or transformation efficiency were observed following Northern and Southern blotting. The enzymatic activities for N-acetylation of two arylamine carcino- gens (2-aminofluorene, 4-aminobiphenyl), and a sulfonamide drug (sulfamerazine) were over 100-fold lower for NAT2 19 compared to reference NAT 4. In addition, activity for O-acetylation of the benzenoid aromatic carcinogen 2-amin- 1-methyl-6-phenylimidazo[4, 5 b] pyridine was 24-fold lower. The results show that NAT2*19 encodes slow acetylator phenotype for both N- and O-acetylation. Our findings define more clearly the effects of individual SNPs on human NAT2 expression, stability and catalytic activity. This work is partially supported by USPHS grant CA34627 from the National Cancer Institute.

A NOVEL REGULATORY MECHANISM FOR TYROSINE HYDROXYLASE

R. R. Vanlancourt and N. A. Sachs. Department of Pharmacology & Toxicology, University of Arizona College of Pharmacy, Tucson, AZ.

Tyrosine Hydroxylase (TH) is regulated by the reversible phosphorylation of serines 8, 19, 31 and 40. The physiological significance of the serine 19 phosphorylation is not obligatory for catecholamine biosynthesis. The purpose of our study was to characterize the physiological relevance of the heterogeneous phosphorylation of TH by identifying novel interacting proteins. Serine 19 is arguably unique due to its requirement of 14-3-3 binding after phosphorylation for optimal enzyme activity, although it has been suggested that phosphorylated serine 40 also binds 14-3-3. 14-3-3 proteins are prevalent in mammalian brain and 14-3-3 isoforms of 14-3-3 serve diverse functions including the regulation of intracellular signal transduction cascades by serving as a scaffold between proteins. To characterize proteins that interact with TH after phosphorylation on serine 19, this amino acid was mutated to alanine (THS19A), since it was reported that a serine to alanine mutation promotes a stable interaction between proteins. For the identification of proteins that interact with TH, THS19A was used as the bait in the yeast two-hybrid system and two cDNA clones were identified, consisting of amino acids 147-262 of mouse-derived PITSLE. PITSLE is a serine/thre- one kinase and characterization of the corresponding human cDNA, in the form of a GST fusion protein, encoding amino acids 1-373 of a splice variant of the 1-373 isoform of human PITSLE demonstrates an interaction between TH and GST- PITSLE (1-373). Additionally, in vitro transcribed and translated TH interacts with full-length FLAG-PITSLE immunoprecipitated from COS 7 cells. TH does not serve as a substrate for PITSLE in an in vitro kinase assay. However, PIT- SLE demonstrates the interaction between TH and 14-3-3 in HEK 293 cells through a mechanism, which remains to be characterized. It appears that PITSLE may serve as a negative regulator of TH activity and therefore, possibly catecholamine biosynthesis.

IDENTIFICATION THROUGH GENE EXPRESSION OF A TOXIC RESPONSE IN THE RAT FOR A COMPOUND THAT EXHIBITS OVERT TOXICITY IN HUMANS, BUT NOT IN RATS.


The classical measures of toxic response have been found to be insensitive to certain known hepatocarcinogens, such as those that cause damage in humans, but whose effects are not detectable within the mammalian species used in pre-clinical trials. The implementation of a more sensitive measure of the toxic response to identify this type of compound is needed to assess accurately the potential toxicity of compounds in humans. For a variety of reasons, gene expression measurements from animal models may provide the means necessary to determine the actual toxic response observed in humans. Tissue is one such compound where an overt hepatotoxic response is observed in humans, but not in pre-clinical animal models. To identify the genes that characterize a response in the absence of overt hepatotoxicity, tissue was administered to Sprague-Dawley male rats and liver tissue was isolated at various time points post-exposure. Using the Affymetrix Rat RUG_34 GeneChip® set as a platform of gene expression levels, we have identified rat genes that are strong indicators of a toxic response for tetrac, but not the vehicle-treated controls. In addition, these genes have been previously shown to be good collective indicators of toxicity using samples extracted from rats treated with...
with known hepatotoxins including acenomphen (APAP), carbon tetrachloride (CCL₄), dibenzofuran, and alpha-naphylsulfonohydrin (ANIT). These results may indicate that marker genes in the liver are detected by administration of known toxicants is more sensitive for detection of the toxic response than classical methods. This expression information can also be used to identify toxic compounds that would otherwise be missed as part of the pre-clinical testing process.

1450

CHROMIUM-INDUCED CHANGES IN GENETIC EXPRESSION IN HEPG2 CELLS, AND IN WINTER FLOUNDER AND MICE LIVER USING MACROARRAYS.


Biological Sciences, University of Texas at El Paso, El Paso, TX and 'Biology, Anderson College, Anderson, SC.

Chromium is the dominant toxicant found at several Superfund sites around the United States, including sites in Odessa, TX and a estuarine site in Maine and South Carolina. However, chromium's effects are not well understood. Therefore, we have attempted to characterize the effects of Cr(III) and Cr(VI) on genetic expression in HepG2 cells, as well as male winter flounder (Pseudopleuronectes americanus), and male CD-1 mice. HepG2 cells were exposed to Cr(VI), as chromic oxide and Cr(III), as chromium chloride hexahydrate to determine their toxicity. Cr(VI) caused death of 80% of the HepG2 cells at 1 μM, while Cr(III) did not cause cell death, but significantly decreased growth at 100 μM. Therefore, cells were treated with 20 μM Cr(III), RNA was extracted and an array was incubated and analyzed. Several genes showed altered expression due to Cr(III), including β-1 antichymotrypsin, cathespin D, and C1. 1-PCR was performed to confirm differential expression of several of these genes. Additionally, mice and winter flounder were examined as in vivo models. Winter Flounder was injected with Cr(III) and Cr(VI) at 400 and 250 μg/kg, respectively, and euthanized 24 hours later. mRNA was extracted from liver and subjected to hybridization was performed to enhance the production of differentially expressed clones, that were amplified by PCR and used to produce macroarrays. The macroarrays were used to confirm differential expression, and clones demonstrating altered expression were sequenced. Additionally, mice were treated with Cr(III) in the water for 14 days at 1000 and 2000 ppm, which approximate 7-5-15 μg/kg/day. Superoxide dismutase was increased in treated mice, suggesting Cr(III) caused oxidative stress. Microarrays will also be performed on mRNA extracted from mouse livers to determine if similar genes are induced in mice, humans and winter flounder by Cr(III).

1451

DEVELOPMENT OF A NOVEL METHOD FOR ANALYSIS OF TRANSCRIPTIONAL CHANGES IN TRANSITIONAL EPITHELIUM FROM URINARY BLADDER OF RATS EXPOSED TO DRINKING WATER DISINFECTANT BY-PRODUCTS.

D. C. Wolf and S. D. Hester. Environmental Carcinogenesis Division, NHEERL, ORD, USEPA, Research Triangle Park, NC.

Epidemiologic studies in human populations drinking disinfected water have consistently shown an increase in the risk of urinary bladder cancer. Increased incidence of transitional cell carcinoma of the urinary bladder is associated with consumption of chlorinated surface waters. The transitional cell epithelium is therefore the target for chemically-induced toxicity and carcinogenicity in the urinary bladder. After chronic treatment to MX alone or a mixture of DBP's, transitional epithelial cells had marked anisokaryosis and megalokaryocytes associated with focal hyperplasia. To detect and analyze biologic events which contribute to transitional cell carcinoma, we developed a method by which the target cell population was removed from the urinary bladder of male rats and the RNA isolated for transcriptional analysis using the Clontech™ Rat Atlas 1.2. Twelve male Long-Evans rats, 4 per group, were exposed to a mixture of potassium bromate, MX, chloroform, and bromodichloromethane administered in drinking water in a mixture of low doses of 0.02, 0.005, 0.04 and 0.07 μg/L, or as a mixture of high doses of 0.4, 0.07, 1.8 and 0.7 μg/L, respectively. In the present study, animals were exposed to deionized water or the mixture solutions for 3 weeks, then euthanized and necropsied. At necropsy the urinary bladder was instilled with 500 microliters of Trizol™ reagent, set for 7 minutes, then flushed to remove the lining cells. The amount of total RNA extracted per bladder after instillation treatment ranged from 2 to 363 micrograms. Total RNA was used to perform the cDNA array analysis which was then verified using real-time PCR. The method will allow study of the biology of the transitional epithelium of the urinary bladder and detection of altered gene expression for improved understanding and defining of biologically relevant responses to urinary bladder carcinogens. This abstract does not reflect EPA policy.

1452

ALTERATIONS IN GLOBAL GENETIC EXPRESSION INDUCED BY AFLATOXIN B1 IN YEAST CYTOCHROME P450 1A2.


Aflatoxin B1 (AFB1) is a human hepatotoxin and hepatocarcinogen produced by the mold Aspergillus flavus. In human, AFB1 is bioactivated by cytochrome P450 (CYP) enzymes, mostly CYP1A2, to a genotoxic metabolite which forms guanine DNA adducts. To characterize the transcriptional responses to AFB genotoxic insult, S. cerevisiae strain Y112, engineered to express human CYP1A2 or vector, was grown to a density of 5 × 10⁶ cells/ml and exposed to AFB (10, 25, 50, 100 μM) or vehicle control (DMDSO) for 2 or 4 hours in uracil(-) medium at 30°C. AFB did not result in significant cell death at 2 hr, determined by colony forming ability, but did induce dose and time-related increases in mitotic recombination measured by Trp+ reversion assay. No cell death or mitotic recombination was observed in the vector expressing strain. CDNA was synthesized from total RNA isolated from treated and control strains expressing cells using oligo dT(15) primers and avian myeloblastosis virus reverse transcriptase, which was then chemically coupled with Cy5 or Cy3 fluorescent dye, respectively. The labeled cDNA pools were admixed and hybridized to a yeast microarray spotted with 6, 246 cDNAs, representing the entire yeast genome. Approximately 200 transcripts were changed by at least two-fold with respect to time and/or dose, which included stress response, protein degradation, DNA replication, RNA synthesis and cell cycle regulation genes, as well as approximately 60% with unknown identity and/or function. Expression of 12 genes increased consistently in a time and/or dose-dependent fashion. Decreased expression was observed for about 30 genes at all conditions except the highest dose (100 μM), at which over 150 genes decreased. Cluster analysis suggested a role for the recombination-mediated DNA repair mechanism, as Rad51 and ribonucleotide reductase genes (RRN1, RRN2, RRN3, RRN4) were co-upregulated in a dose- and time-dependent manner. (Supported by NIH grants ES07033 and ES05780).

1453

ALTERED LIVER GENETIC EXPRESSION AND POSTTRANSLATIONAL PROTEIN MODIFICATIONS IN RESPONSE TO LETHAL OR NON-LETHAL LISTERIA MONOCYTOGENES INFECTION IN FEMALE BALB/C MICE.


Infection of mice with L. monocytogenes (LM) is widely-used to study host response to intracellular bacterial pathogens and to examine immunomodulatory effects of therapeutics. LM is also a food-borne pathogen and is thought to act in part by modifying host cell signal transduction and utilization of the host's actin-based cytoskeleton. While this model is well studied, effects on murine gene expression remain largely unknown. The objective of this study was to determine if infection induced differential gene expression or protein modification in the liver, a primary target organ. Mice received by i.v. route either vehicle, 1x10⁸ (non-lethal), or 5x10⁸ CPU LM (lethal) and were sacrificed 1, 2, 4, and 14 days later. Levels of RNA were measured with Affymetrix Genechip® arrays. Differentially phosphorylated proteins were identified using gel separation and MALDI-TOF. Infectious burden, clinical and histopathology confirmed infection and showed significant effects in the high dose group. Multiple genes were found to be differentially expressed in a reproducible and dose-related manner. Classes of genes altered included immune-function genes (lysozyme, cytosine, lymphotoxin), genes associated with liver toxicity (Ntcp, hyposia inducible factor, HSP 70), and genes involved in cell signaling (EGF-R, IC5BP-1, IFG-R-2). In addition, using data analysis and modeling tools we have identified a preliminary set of genes that may be useful in assessing if an infection with LM will be lethal or non-lethal. Protein analysis indicates that, γ-actin, ATP-synthase β chain and CDCS kinase regulatory subunit undergo differential tyrosine phosphorylation. These results indicate large-scale differences in gene expression and protein patterns as well as in protein modification in livers of exposed mice. Supported in part by NIAID Contract N01-AI-05417.

1454

TRANSPLENTAL EXPOSURE TO ESTROGENS INDUCES A DISTINCTIVE GENETIC EXPRESSION PROFILE IN THE FETAL UTERUS OF THE RAT.


In order to understand the molecular events associated with the estrogenicity of different chemicals, we have used microarray technology to determine whether there is a common set of genes which expression profile could be traced to estrogenic com-
1455 Efects of Gestational and Lactational Exposure to Diethylstilbestrol on Testicular Gene Expression Using cDNA Microarrays and Real-Time PCR.
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, and Department of Animal Sciences, Michigan State University, East Lansing, MI.

To explore the molecular events underlying the adverse effects on sperm quality following diethylstilbestrol exposure to environmental chemicals, we have constructed cDNA microarrays to examine testicular gene expression in B6DF2F1 offspring of mice exposed by gavage to 10 μg/kg diethylstilbestrol (DES) from gestational day 12 to postnatal day 21. Replicate gene expression profiles were examined in male offspring at 3, 15, and 45 weeks of age to determine if the adverse effects on sperm quality paralleled the long-term effects on weight, sperm count, and sperm fertilizing ability, as previously described. A mouse cDNA microarray containing approximately 1,488 genes was constructed and used to compare testicular gene expression in the offspring of DES-exposed mice to that of vehicle-exposed mice. An independent reference design coupled with paired t-tests was used to identify genes significantly (p<0.05) altered in expression by DES. Based on adjusted p values, there was only one gene significantly altered at 3 weeks of age (a component of the GST proteasome). At 15 weeks of age, there were 46 genes significantly altered in expression, with no significant changes at 45 weeks of age. Raw p values and functional annotation were used to prioritize the selection of genes for verification. Using a real-time PCR, both age and dose dependence of changes in gene expression were examined. There was a significant decrease in the expression of ER alpha mRNA at 3 weeks of age (p<0.01), while mRNA expression was below the limits of detection in 15 and 45 week-old mice. Other genes selected for verification include the orphan receptor TR2, and Xmn, a component of the synaptic complex. The results suggest that the adverse effects on sperm fertilizing ability may be due to altered expression of ER alpha, and possibly other genes, in the testis.

1457 Microvesicular Steatosis Induced by Short Chain Carboxylic Acid Analog: Evaluation of Gene Expression and Correlation to Mitochondrial Toxicity.

Hepatotoxicity characterised by microvesicular steatosis features abnormal accumulation of numerous small cytoplasmic lipid droplets in hepatocytes. Fulminant or progressive cases may lead to liver failure and death. Experimentally, short-chain fatty acids are known to induce hepatocellular microvesicular steatosis. The precise mechanism(s) whereby short-chain fatty acids induce microvesicular steatosis are unknown, although there is evidence implicating disruption of mitochondrial function. The identification of genes that correlate with microvesicular steatosis could provide a better understanding of how the toxicity occurs. To this end, a series of short chain fatty acids including valproic acid (VPA), cyclopropane carboxylic acid (CPCA), 4-pentenoic acid (4PE), pivalic acid (PIV), butyric acid (BTR) and propionic acid (PRO) were tested in vitro for their ability to inhibit microvesicular beta-oxidation and in vivo for 1, 3, and 7 days to evaluate their ability to induce microvesicular steatosis in rats. VPA, CPDA and PIV resulted in microvesicular steatosis 1-3 days post treatment while PRO and BTR did not. In agreement with this finding these compounds inhibited mitochondrial beta-oxidation in a dose dependent manner in isolated rat liver mitochondria. However, no evidence was evident with PRO or BTR. Gene expression results showed a regulation in genes involved in beta-oxidation, including thiolase, acyl-CoA dehydrogenase and cytochrome P450 2C11, as well as some of the other genes not previously associated with microvesicular steatosis. Overall, these results support altered hepatic mitochondrial function as a causative mechanism of the toxicity induced by short-chain fatty acids and provide potential markers for this toxicity.

1458 Concordance of Degree of Renal Injury with Gene Expression in Individual Animals Treated with the Nephrotoxicant Cisplatin.
K. L. Thompson, M. L. Minsky, and E. D. Sistare. CEER, USDA, Laurel, MD, and Drug Safety Evaluation, Pfizer, Inc., Groton, CT.

As part of a coordinated evaluation of the application of genomic methodologies to mechanism-based risk assessment, the Nephrotoxicity subteam of the IL11 Genomics and Proteomics Subcommitte examined the relationship between traditional toxicologic endpoints and gene expression responses to prototypic nephrotoxic drugs in Sprague-Dawley rats. As part of one study, 5 male rats received a single ip injection of 5 mg/kg cisplatin. Six days later, the animals were sacrificed, and serum chemistry and urinalysis parameters were measured, and the kidneys were processed for histopathology and RNA. Gene expression profiles were evaluated with the 700 gene Phase 1 Rat(C57) microarray on samples from individual animals, pooled controls, and pooled treated animals. Of the 5 animals that had moderate tubular necrosis and regeneration, and highly elevated serum levels of BUN and creatinine, one animal had mild tubular necrosis and regeneration, and no evidence of kidney injury was observed in a fifth rat. Both of these latter animals had normal BUN and creatinine levels. The gene expression induction profiles were highly correlated among the 5 animals that had the same degree of renal damage and altered serum chemistry, and with the pooled sample from treated rats. In a subset of genes, there was a narrow scatter band between the magnitude of the induction and the type and extent of tubular injury seen. The observed gene induction profile was highly concordant with results from pooled animals in an earlier, independent study using the same dose of cisplatin. Genes significantly up-regulated in animals with evidence of renal toxicity included damage-inducible genes like CADD13, clauterin, and cyclin G. Renal tubular markers like OAT3, HNF4, and EGF were
significantly down-regulated in these animals. The observed patterns of gene induction in this study were consistent with known mechanisms of cisplatin nephrotoxicity, highly reproducible, and reflective of the extent of damage observed in histopathologic analysis.

1459 TEMPORAL EXPRESSION PATTERNS OF GENES IN THE UTERI OF IMMATURE, OVARIECTOMIZED MICE TREATED WITH ETHYNYL EstrADIOL.

K. C. Ferrush1, J. Ecker2, C. Gerrings2 and T. R. Zacharewski1. 1Department of Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, and Institute of Environmental Toxicology, Michigan State University, East Lansing, MI and 2Department of Biostatistics, Virginia Commonwealth University, Richmond, VA.

Estrogens are classically defined as compounds that are capable of inducing a trophic effect on the uterus. This physiological change has been linked to alterations in the expression of estrogen-responsive genes, but the cascade of molecular events remains unknown. In the present study, the effects of ethynyl estradiol (EE), a synthetic estrogen, on global gene expression in immature uteri were examined in intact, ovariectomized C57BL/6 mice. Mice were gavaged with 0.1 mg/kg EE or vehicle, and uteri harvested at 0, 2, 8, 12, or 24 hr. Overall, while no differences in uterine weight were found between treatment groups (p=0.296) from 0 to 24 hrs, changes in global gene expression levels were observed during this period.

MuTread Affymetrix GeneChips were used to assess relative expression levels of over 10,000 genes in uterine tissue of two groups from each treatment and time-matched vehicle treated (control) group. Data were then screened using standardized t scores to identify gene expression changes outside the normal range of variation for the control group. Treated values were standardized using (1) a global control mean and standard deviation assuming no time effect among the control observations, or (2) the average and standard deviation of the time-matched control values. These methods were found to give different results, with the time-matched control approach resulting in the identification of fewer active genes. Large t scores were interpreted to indicate active gene responses. Overall the 24 hr time point had the largest t scores as determined by average ranks and summary statistics over time. The nature of the changes over time was characterized using a parametric model based on the general shape of the time-effect relationship.

1460 EVALUATION OF DIFFERENTIAL GENE EXPRESSION IN THE SPLEENS OF RATS AT SEVERAL TIMEPOINTS AFTER INJECTION OF CYCLOPHOSPHAMIDE.

Y. Ng-Smith, K. W. Tiegler, J. T. Gonzales and G. M. Farris. Database, PHASE-1 Molecular Toxicology, Santa Fe, NM.

The toxicity of drugs and chemicals is dynamic with overlapping stages of drug metabolism, direct and indirect cellular damage, and repair. Recognizing this complexity in the toxicity response, phase 1 evaluates gene expression by sacrificing rats at 6, 24 and 72 hours after a single intraperitoneal injection of a compound at two concentrations. For this study Sprague-Dawley rats were dosed with several immunotoxicants. Cyclophosphamide dosed at 25 and 100 mg/kg induced lymphoid depletion in the spleen at 72 hours. The spleen of each rat was harvested and total RNA was isolated for transcription to cDNA with dual cy-3, cy-5 labeling and hybridized on the PHASE-1 rat 700 gene toxicology array. The gene expression levels were compared across time points and across doses. The differential gene expression with cyclophosphamide was also compared with other immunotoxicants. The ability to detect toxicity by determining differential gene expression improves the process of evaluating toxicity in pre-clinical studies with rats.

1461 THEOPHYLLIN INDUCED DIFFERENTIAL GENE EXPRESSION IN THE HEART.

D. B. McGinn, G. M. Farris and C. L. Reynolds. PHASE-1 Molecular Toxicology, Santa Fe, NM.

Gene expression data generated using PHASE-1’s comprehensive toxicological, 700-gene microarray was evaluated in order to compare gene expression profiles for different cardiac toxicants. Labeled cDNA was produced by reverse transcription of mRNA isolated from tissue harvested at 6, 24, and 72 hours after rats received a single intraperitoneal injection of a cardiac toxicant at two concentrations. This cDNA was then hybridized onto arrays composed of genes empirically determined to be toxicologically relevant. The gene expression showed correlations between theophyllin (cyclic phosphodiesterase inhibitor) dosed at 25 and 100 mg/kg, and other cardiac toxicants, including nifedipine (calcium channel blocker) dosed at 30 and 120 mg/kg. The data corresponded to the histologic evidence of myocardial degeneration and inflammation observed at 72 hours and to the compound’s mechanism of toxicity. This study demonstrates how microarray technology used at PHASE-1 can be a powerful toxicological tool.

1462 SEXUAL DIMORPHISM OF HEPATIC GENES EXPRESSED IN HEXACHLOROBENZENE (HCB)-TREATED RATS.

B. Ali, M. Charbonneau and D. G. Crym. Human Health Research Centre, INRS-Institut Armand-Frappier, Pointe Claire, PQ, Canada.

HCB is an epigenetic carcinogen that predisposes females to the formation of liver tumours. Short-term exposure (5 days) to HCB promotes, with greater efficiency in females than in males, liver tumours induced by DEN injection 95 days following the last treatment. The objective of this study was to determine gender-specific differences in hepatic gene expression and their relationship to tumour promotion. To address this objective, four experimental groups of rats were used: 1. vehicle-exposed males (control); 2. vehicle-exposed females (controls); 3. HCB-treated males; 4. HCB-treated females. Rats were administered corn oil (vehicle) or HCB (100 mg/kg) by gavage for 5 consecutive days and sampled on day 50 of the experiment. Cellular mRNA was isolated from the liver of each rat (n=4/group) and used to screen an Atlas array 1.2 Il (Clontech) containing 1776 genes. Results indicate that in control male rat liver, 137 genes could be detected using this array, with 94% of expressed hepatic genes being common to each individual. In the female livers, 186 genes were expressed and 82% of these were common to each individual. These results indicate that, of the genes screened, there are 26% more genes expressed in female livers than in males and that there is more variation in the expression of these genes between individual females than between males. In HCB-treated males, there were 161 genes expressed, i.e. an 18% increase in the number of genes expressed as compared to controls, but only 71% of these were expressed in all four males. In HCB-treated females, 153 genes were expressed, i.e. an 18% decrease in the number of genes expressed as compared to controls, and 83% of these were found in each individual female. Male versus female differential comparisons of HCB-modulated genes will be discussed in relation to gene clusters that may be responsible for the gender-specific response in liver tumour promotion. (Supported by TSRI, Health Canada)

1463 QUANTITATIVE PROFILE OF TESTICULAR GENE EXPRESSION PATTERNS IN MICE EXPOSED TO DIETHYLSILBASTROL DURING GESTATION AND LACTATION.

P. Saam1, M. Fielder1, J. Han1, S. M. Samy2, T. Zacharewski1 and K. Chou1. 1Animal Science, Michigan State University, East Lansing, MI; 2Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI and 3Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.

Previously, we have reported decreases in sperm fertilizing ability in male offspring exposed to diethylstilbestrol (DES) during gestation and lactation. In this study, a mouse cDNA microarray containing approximately 1948 genes was used to profile the testicular gene expression of the offspring. Pregnant C57BL/6 female mice bred with DBA/2 males were gavaged with 10 ug of DES/kg of B.W./day from gestational day 12 to postnatal day 21. The construction of cDNA microarray and gene expression identification by comparison of statistics and P-values have been reported in a separate presentation. In this report, the fluorescence measurements were analyzed using two interconnected mixed models, a normalization model and a gene model. The normalization model accounted for experiment-wide systematic effects that could bias inference made from the data from individual genes. The residuals from this model represent the normalized/s values, and were the input for the gene model. The gene model was fitted separately to the normalized data for each gene, allowing inferences to be made using separate estimates of variability. Differences between Least Squares Means for treatment groups were estimated using Scheffe adjustments. At 15 weeks of age, the expression of 20 genes in the exposed males were four times or higher of the levels in the controls. Additional 39 genes were expressed at least twice of that in the controls. Among the genes which expression was suppressed, 31 genes were expressed less than one fourth of that in the controls and another 31 were expressed at less than half of the control levels. Over expression of prostatic receptor, serine/threonine kinase 10, and tyrosine phosphatase under expression of prostaglandin E receptor 1 are among the several prominent changes observed in the testes of DES exposed offspring.

1464 IDENTIFICATION OF DIFFERENTIAL EFFECTS OF BENZ0(A)PYRENE AND 2, 3, 7, 8- TETRAChLOROBENZO(p)-DIOXIN ON GENE EXPRESSION IN HUMAN PLACENTAL JEG-3 CELLS USING CELL CYCLE AND DNA MICROARRAY ANALYSES.


Maternal cigarette smoking disrupts placental growth and function, and the aryl hydrocarbon compound benzo(a)pyrene (BaP), a major toxicant in cigarette smoke, has been shown to inhibit proliferation and growth factor expression in cul-
tered human placental cells and JEG-3 chorioncarcinoma cells. This study further investigated the effects of BaP on cell cycle and gene expression in JEG-3 cells in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the reference ligand for the aryl hydrocarbon receptor (AhR), and benzo(a)pyrene (BaP), a weak ligand for the AhR. JEG-3 cells treated with 10 μM BaP for 48 hours showed a 75% decrease in thymidine incorporation, whereas 10 nM TCDD had no effect. Flow cytometry analysis showed that BaP induced a G2/M cell cycle arrest, whereas TCDD and 50 μM BaP had no effect on cell cycle phase distribution. Cell cycle regulatory protein expression was determined by Western blot and immunohistochemistry. BaP-mediated induced expression of nuclear p21 protein, a major inhibitor of cyclin-dependent kinases. Although BaP had no effect on total cellular p53 levels, phosphorylation of p53 at serine 15 was markedly increased. In contrast, neither TCDD nor BaP had any effect on the expression of p21 or p53 phosphorylation.

Lastly, a human cancer cell line was used to evaluate changes in gene expression induced by treatment with BaP and TCDD. Data were analyzed using Atlas Image, Cluster and Treeview programs. BaP down-regulated 7 genes, while 3 genes were up-regulated. In comparison, TCDD was found to down-regulate 9 genes, while 1 was up-regulated. Of these, BaP and TCDD had similar effects on only 3 genes. In summary, although BaP and TCDD are both ligands for the Ah receptor, only BaP produces G2/M cell cycle arrest with induction of p21 and p53 serine phosphorylation that is characterized by a unique pattern of gene expression in JEG-3 cells. (Supported by NIH ES07375)

1465 DIFFERENTIALLY EXPRESSED SEQUENCES IN THE MUMMICHOCH AS A RESULT OF ANTHOCHEMICAL EXPOSURE.

J. S. Peterson and L. L. Bain, Dept. of Biology, University of Texas at El Paso, El Paso, TX.

Approximately 40% of all USEPA Superfund sites have some sort of polycyclic aromatic hydrocarbon (PAH) contamination. Many of these sites are scheduled for remediation and tools are needed to assess whether efforts have been successful. Knowledge of the induction or suppression of specific genes as a result of exposure may be useful in understanding the mechanisms of PAH toxicity. We are attempting to generate a fingerprint of exposure to the PAH anthracene using the differential-display reverse transcription polymerase chain reaction (DD RT-PCR) technique. This technique has produced a series of cDNA fragments that will be used in constructing a microarray specific to anthracene. We then compare these fragments that have been detected to sequences of known genes in order to identify potential physiological consequences of exposure to anthracene. Mummichogs (Fundulus heteroclitus) are a non-migratory estuarine fish used to model exposure because they have contact with contaminated sediments over the full course of their lifetime. The fish were subjected to 7-day static renewal tests using environmentally relevant concentrations of 0, 27, 50 ppb, and 80 ppb of anthracene. The fish were euthanized, livers were removed, and total RNA was extracted. The mRNA was reverse transcribed into cDNA, amplified by randomly designed primers and run on a differential display gel. Sixteen cDNA fragments that represented differentially expressed mRNA sequences were isolated, amplified, and sequenced. The GENBANK database was searched to compare these mummichog sequences to known genes of known function. Three sequences that have shown similar sequence to known genes—CYP212 in the mummichog, insulin-like growth factor 1 in the Japanese flounder (Paralichthys olivaceus), and tryptophan in both the Atlantic cod (Gadus morhua) and the Mami cod (Vanrosbathra magellanea). Future work will relate the up- or down-regulation of these genes to the physiological impacts of anthracene exposure.

1466 THE LONG-TERM EFFECTS OF NEONATAL EXPOSURE TO ENDODUCK DISRUPTORS ON TESTICULAR GENE EXPRESSION IN MICE.

M. Komiyama, T. Adachi, and C. Mori, Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan and Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Kawagushi, Japan. Sponsor: A. Puga.

In order to evaluate the feasibility of cDNA microarrays for the risk assessment of endocrine disruptors (EDs), alteration of gene expression profiles was analyzed in adult mouse testes after neonatal exposure to diethylstilbestrol (DES), bisphenol A (BPA) or genistein (Gen), using cDNA microarrays. Analysis with Mouse GEM revealed that expression levels of 34, 38 and 12 genes were changed in response to DES, Gen- and BPA-treated mice, respectively. Gene expression profiles were very similar between DES- and Gen-treated mice, but that of BPA-treated mice was quite different. These results suggest that gene expression profiles might be feasible for grouping of EDs in terms of their effects. Further, it is suggested that mechanisms of BPA action may be different from those of DES and Gen, although they are all estrogen-like compounds. Next, we investigated transition of the expression profiles in testes of DES treated mice during 4-12 weeks of age using in situ cDNA microarrays. Many of genes affected by DES were up- or down-regulated at restricted periods, but some genes were continuously up-regulated or shifted from down-regulated state to up-regulated state. These results suggest that the transition patterns may represent the gene expression cascades that were affected by EDs. Moreover, the genes that were up-regulated continuously in the long term might be a candidate for the biomarker indicating that the exposure of individuals to EDs occurred at some sensitive period. We propose that cDNA microarray is a useful tool, which provides us a bird’s eye view of global effects of EDs on the gene expression.

1467 EFFECTS OF DIETARY EXPOSURE TO GENISTEIN AND METHYXOCHELIRIN ON THE GENE EXPRESSION PROFILE OF THE MAMMARY GLAND IN MALE RATS.

E. J. Bartolucci and L. Yao, Systems Biology, CHET Centers for Health Research, Research Triangle Park, NC.

Our previous study has shown that the isoflavone genistein (GE) altered the toxicological effect of the pesticide methoxychlor (MXC) on the growth pattern of mammary gland in male rats. The objective of this study was to determine the gene expression profile in the mammary glands of GE- and MXC-treated male rats. Sprague-Dawley rats were fed a soy- and alfalfa-free diet containing GE (800 ppm) and MXC (800 ppm), either alone or in combination throughout pregnancy and lactation. Dietary exposure of the offspring was continued until postnatal day 90, when the pups were killed. Total RNA was isolated from one inguinal mammary gland of each non-littermate (n = 3). Radiolabeled cDNA was generated using Clontech CDS primer labeling and hybridized to Clontech Rat 1.2 arrays with (1, 176 gene probes). Phosphomager-detected radio signals were background-adjusted (Atlas Image, Clontech) and analyzed for gene expression patterns (GeneSpring, Silicon Genetics). Both the GE and MXC treatments altered the gene expression profile of the mammary glands. More genes were affected (at least three-fold up- or down-regulation) in the treated groups than in the control group when data values were normalized to the 50th percentile of all the measurements and then to each gene itself. The combination of GE and MXC showed a more prominent effect than either compound alone. Some subsets of genes were only affected in the GE group, only in the MXC group, and only in the combination group (47, 47, and 172 genes, respectively), while 78 genes were affected in all treatment groups. The general pattern of the changes is in close agreement with treatment effects observed at the morphological and biochemical levels. While the toxicological significance of such changes in gene profiles is not yet clear, we expect that further analysis of this data will help guide our research on elucidation of the pathways leading to the GE- and MXC-induced mammary gland effects.

1468 THE USE OF DNA MICROARRAY TECHNOLOGY TO ANALYZE THE EFFECT OF 3, 4- DICHLOROPROPIONANILIDE (DCPA) ON HUMAN T LYMPHOCYTES.


DCPA or propamine is the active ingredient in post-emergence herbicides such as WHAM! E/Z/Band is used extensively in the cultivation of rice. Previous work in our laboratory using Both an in vivo mouse model and an in vitro exposure model has demonstrated that DCPA inhibits several important immune system functions including both T-independent and T-dependent antibody responses, cytokine production and natural killer (NK) cell function. Additional studies using the human T lymphoma cell line Jurkat have demonstrated that exposure to 100 μM DCPA decreased the secretion of IL-2. The decrease in IL-2 production is due in part to a decrease in the DNA binding activity of the transcription factors AP-1, NFAT and NF-κB. To understand the overall effect DCPA has on human T lymphocytes DCPA microarray technology was employed. Jurkat cells were stimulated with PMA and the calcium ionophore A23187 and exposed to 100 μM propamine or ethanol (vehicle control). The experiment was designed to address three questions: 1) what are the initial or early (15 and 30 min) effects of DCPA exposure? 2) What are the downstream (2 and 8 h) effects? 3) What are the long-term (24 and 36 h) effects? Using the TostChip created by the NIEHS Microarray Center, we analyzed the mRNA expression patterns of approximately 2000 genes. Results from two independent experiments demonstrated that 92 genes or 4.6% of the genes analyzed were affected by DCPA exposure in each experiment. Some of the affected genes included transcription factors, cell cycle control genes, kinases, transcription/translation genes and phosphatases. Consistent with our previous data, many of the genes whose mRNA expression was decreased in DCPA treated cells are known to be regulated by at least one of the three transcription factors AP-1, NFAT and NF-κB which have decreased DNA binding activity in DCPA treated cells. Supported by NIEHS ES07512
Tumor necrosis factor alpha (TNF-α) is an important cytokine with pleiotropic effects on cellular functions. TNF-α can mediate cellular responses either in a paracrine or autocrine manner by activating the signaling pathways involved in the expression of appropriate genes. It is well documented in the scientific literature that under certain in vitro conditions, TNF-α can quickly and markedly induce the expression of early response genes. In order to investigate the rapid effects of TNF-α, we exposed a macrophage cell line, RAW 264.7 cells, for 1 hour to exogenous TNF-α. In two identical experiments we identified 147 cDNAs that were consistently induced >2 fold. Many of the induced genes fell into 3 categories: cytokine and immune function (TNF-α, macrophage colony stimulating factor, small inducible cytokine A2, immune responsive gene 1, IL-15 receptor, CD 48 antigen, TNF-α stimulated ABC protein); cell signaling and transcriptional regulators (p38, zinc finger like protein 36 (zfp36), c-myec oncogene, lck like receptor, guanine nucleotide binding protein alpha, serum-inducible kinase, fos-like antigen 2), and lipid metabolism (very low fatty acyl CoA synthetase and cytochrome P450 1A1). Interestingly, among the 147 consensus genes induced by TNF-α, the most highly induced were TNF-α itself (>25 fold) and zfp36 (>25 fold). zfp36 is a transcriptional factor known to modify the stability of TNF-α mRNA. Overall, the genes induced are consistent with a known physiological role of TNF-α in amplifying the immunomodulatory response. Currently, studies are ongoing to better understand and tease out the specific TNF-α signaling pathways involved in autocrine regulation of macrophage function.

Identification and characterization of genes responsive to perfluoroctanoate sulfonic acid exposure utilizing differential display and gene chips.


Differentiation display, first introduced by Liang and Pardee (1992), is a useful method to identify differences in gene expression at the whole genome level. Affymetrix rat genome U34A chip, containing ~8000 full-length annotated genes and EST clusters, has been proven to be a powerful tool in toxicology studies using rat as model system. Perfluoroctanoic sulfonic acid (PFOS) is the end metabolite of a number of perfluorinated fatty acids analogues extensively used in industrial materials and commercial applications. Few studies have been conducted on this novel compound, and its mechanism of action still remains unclear. In the current study, the restriction fragment differential display (RFDD-PCR) a modified version of the original method and Affymetrix gene chips were used to identify alterations in gene expression levels due to PFOS exposure in vitro and in vivo. RFDD PCR was used to assess liver hepatoma cells were treated with solvent control, PFOS at 10 and 50ppm in medium for 96hrs. SD rats were oral gavaged with vehicle or PFOS at 5mg/kg/day for either 3 days or 3 weeks. RNA samples were extracted from cells and rat livers and prepared for subsequent analysis. Following the RFDD-PCR procedure, 90 bands on the sequencing gel were identified as different among treatment groups. All these candidate genes were sub-cloned and sequenced. GeneChip analysis was conducted by hybridizing U34A chips with experimental samples, and analyzed using microarray suite software and data mining tools. Genes that were differentially expressed in response to PFOS exposure can be clustered as genes coding for fatty acid metabolism, nuclear binding factors, and proteins involved in signal transduction pathways. Consistent results were obtained from replicates in gene chips, however, expression profiles in vitro and in vivo showed only limited similarity.

The hypoxia inducible factor, HIF-1α, is critical to metal induced toxicity.

A. Vengellur 1 and J. L. LaPette 2, 1 Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI and 2 National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI. Sponsor: J. Trusko.

Metal induced toxicity is one of the most important environmental health issues facing humans. Metals, such as nickel, cobalt, lead and cadmium are known or suspected carcinogens. The mechanism of action for the toxicity of these metals remains largely unknown. The process has been linked to a number of factors, including stress proteins, reactive oxygen species, DNA methylation and others. To identify target genes for metal induced toxicity we utilized DNA microarray representing approximately 9000 human genes. Hep3B cells were treated with nickel (100 μM), cobalt (100 μM) or hypoxia (1% O2) for 24 hours. Hypoxia treatments lead to significant expression changes in 61 genes. These genes included hypoxia regulated genes (e.g. vascular endothelial growth factor and adrenomedullin) and several new hypoxia regulated genes. In addition, cellular iron (HIF-1α suppressor protein and the WSB-1 signaling protein). Greater than 50% of these hypoxia regulated genes were also regulated by nickel and/or cobalt. The overlap between these expression profiles suggests a common signaling system. The most likely target for this central mediator is the hypoxia inducible factors (HIFs). To test the hypothesis mouse embryonic fibroblasts (MEFs) from wild type and HIFα null (HIFα-/-) animals were prepared. These two cell types were treated with various concentrations of cobalt and their growth properties were analyzed. In wt MEFs, cobalt exposure (100 μM CoCl2) displayed significant toxicity. Growth rates were decreased by 75% when compared to the untreated controls. The HIFα-/- MEFs, however, showed little if any toxicity at the concentration. To determine the potential targets of the metal induced toxicity cDNA microarray experiments were performed on several cultures of the wild type and null MEFs. Preliminary results suggest a subset of genes is differentially regulated in the wt cells. These results suggest that HIFα plays an important role in metal induced toxicity.

Geneichi microarray analysis provides insight into the disruptive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on urogenital sinus (UGS) and prostate development.

T. M. Lin, U. Simamane and J. E. Peterson, School of Pharmacy, Univ. of Wisconsin, Madison, WI.

Mouse prostate development starts as projections (bud) of UGS basal epithelial cells into the surrounding mesenchyme on Gestation Day (GD) 16. In utero TCDD exposure blocks onogenesis of ventral buds, reduces dorsal bud number, and delays anterior and dorsolateral bud formation. To investigate the mechanism by which TCDD disrupts UGS development, we examined gene expression in the UGS. (Pregnant C57Bl/6j) mice were given 5 μg TCDD/kg or vehicle on GD 13. On GD 16, UGSs from male fetuses were separated into ventral and dorsal mesenchyme and epithelium. Total RNA preparations were pooled to make one composite sample per tissue and treatment group. Probes were generated and hybridized onto Affymetrix Murine Genome U74A and U74Av2 arrays. Using Microarray Suite software, 195 genes were identified whose steady state mRNA levels were altered by TCDD in at least one region of the UGS. Forty-five of these ESTs were unknown. Forty-one genes belong to cytok fascism and extracellular matrix families. Cell adhesion molecules involved in skin development were also altered, suggesting a major disruption of UGS epithelial development/organization. Twenty-nine altered genes (neutral specific intracellular structure, neural crest markers, and neuropeptides) are associated with neural development. TCDD-AHR targets gene were also activated. Five transcription factor genes were altered, of which HNF-3 and capsule were involved in prostate development. Only 7 altered genes belong to growth factor signaling pathways, of which only TGFβ is known to be important to prostate development. Growth factors may not be important at this earlier stage of UGS development, or spatial distribution may be more important than quantity. Preliminary results from real time LightCycler RT-PCR support the GeneChip results. These findings enhance our understanding of TCDD disruption of fetal UGS development and suggest new research directions, e.g. neural crest cell distribution and function. (NIH Grant ES10332 and Fullbright Grant)

Differentiation between sensitizers and irritants in the local lymph node assay by global expression profiling.

P. M. Collier 1, G. E. Hollis 1, T. C. Burn 1, B. D. Carl 1, W. R. Foster 2, R. M. Huber 3, C. Smith 1, C. M. Glatt 1 and G. S. Lade 1, DuPont Pharmaceuticals Co, Newark, DE and 2DuPont Co, Haskell Laboratory, Newark, DE.

The objective of this study was to use the local lymph node assay (LLNA) in conjunction with high density DNA microarrays to 1) evaluate expression profiling as a technique to discriminate between structurally diverse sensitizers and irritants; and 2) identify potential transcript biomarkers specific for each compound class. Expression profiling was performed on auricular lymph node RNA isolated from female CBA/J mice (n=8/group) dosed with either 4% aceone/olive oil (vehicle control) or one of four sensitizers (0.25% dimethylchlorobenzene, DNCB; 0.05% oxazalone, OX; 25% trimethylamine N-oxide, TMA; 1% tolune disocyanate, TDI) or one of four irritants (25% chlorobenzene, CB; 5% phenol, PH; 20% boric acid, BA; 0.5%, 1%, or 2% benzalkonium chloride, BAC) according to the standard LLNA protocol. All experiments were performed using 12,000-gene Affymetrix arrays. Two-dimensional agglomerative cluster analysis revealed a statistically significant similarity in the expression profiles of sensitized-treated animals (R2 > 0.6; P < 0.01). Irritant profiles clustered together for an unrelated group with no correlation to the sensitiser cluster (R2 < 0.8). Evaluation of sensitiser-specific sig-
natures revealed a strong induction of multiple cell cycle and inflammatory response transcripts that are unaltered by irritant treatment. These clusters of potential biomarkers effectively confirm proliferation-based scoring of sensitizers in the LLNA. Expression profiling performed on higher doses of BAC that typically score positive in the LLNA revealed a similar induction of many of these same cell cycle and inflammatory-response transcripts, providing a molecular basis for high-dose irritant scoring in the LLNA. These results illustrate the utility of queryable expression profile databases for compound classification, and revealed several candidate biomarkers that may serve as sensitive endpoints for the LLNA.

DNA ARRAY ANALYSIS OF GENE EXPRESSION ALTERATIONS UNDERLYING SENSITIZATION USING THE LOCAL LYMPH NODE ASSAY AS A MODEL.
C. M. Glatt, C. Smith, C. S. VanPelt and G. S. Ladies. The DuPont Company, Newark, DE.

The objective of this study was to identify the underlying and distinctive genetic mechanisms of dermal and respiratory sensitization using the local lymph node assay (LLNA) and DNA array technology. We have previously shown that there are temporally dependent alterations in gene expression in mouse auricular lymph nodes at days 1, 3, and 6 of dosing with a known dermal sensitizer, 1-chloro-2,4-dinitrobenzene (DNB). The current study was designed to (1) examine early gene expression changes following exposure to DNB, as well as a known respiratory sensitizer, trimellitic anhydride (TMA); and (2) identify markers to discriminate between dermal and respiratory sensitization. Female C57Bl/6J mice were dosed with either 4% acetone oil (AEO) vehicle-control, 0.25% DNB, or 25% TMA. Groups of vehicle-control (n=8), DNB-treated (n=8), and TMA-treated (n=8) mice were sacrificed and auricular lymph nodes (AULNs) were removed and pooled within each group 1, 4, 12, and 24 hours following a single dose of either DNB or TMA. An additional group of nine (n=9) mice were sacrificed and AULNs were removed and pooled at the 24-hour time point. Total RNA was isolated and cDNA was prepared and radiolabeled using gene-specific primers to probe a murine cDNA expression array (Atlas Mouse 1.2 Array; Clontech Labs, Inc.). Of the arrayed genes, DNB-induced changes in expression levels were detected in approximately 0.8%, 0.3%, 3.4%, and 0.2%, and TMA-induced changes were detected in 1.2%, 1.1%, 3.1%, and 1.8% at 1, 4, 12, and 24 hours, respectively. Unregulated gene expression signatures between DNB and TMA were revealed. These data demonstrate the differential and temporally dependent gene regulation that occurs following the topical application of a known dermal or respiratory sensitizer. Additionally, these data illustrate the utility of expression profile databases to distinguish dermal from respiratory sensitizers that are traditionally indistinguishable in the LLNA.

DNA MICROARRAY ANALYSIS OF GENE EXPRESSION DURING CHEMICAL INDUCED NEPHROCARCINOCENICITY IN THE EKER RAT.
S. K. Patel, J. L. Monks and S. S. Lau. Center for Molecular and Cellular Toxicology, University of Texas at Austin, Austin, TX.

The Eker rat, derived from the Long-Evans strain, provides an excellent model for studying renal carcinogenesis. A germ-line insertion in the tuberous sclerosis (Tie-2) tumor suppressor gene predisposes these rats to the development of spontaneous or chemical induced tumors. Hydroquinone (HQ) is a rodent carcinogen and a possible human carcinogen. 2, 3, 5-trihydroxy-2-methylphenoxy (TGHQ) has been implicated as a reactive metabolite that contributes to HQ-mediated nephrocarcinogenicity. The mechanisms of HQ-mediated renal carcinogenesis remain unknown. TGHQ-treated rats develop numerous toxic proximal tubular dysplasias that represent early transformation within tubules, and adenomas are seen to arise from these lesions. cDNA microarrays were used to examine aberrant gene expression associated with TGHQ-induced transformation in tumor tissue derived from the kidneys of Eker rats treated with TGHQ. The expression of 38 genes were altered by three-fold or greater in tumor tissue compared to normal kidney tissue. Furthermore, the levels of a number of genes, identified using cDNA microarrays, were validated by semi-quantitative RT-PCR. The differentially expressed genes are involved in cell cycle regulation (cyclin-dependent kinase 4), signal transduction (extracellular regulated kinase), stress response (glutathione-S-transferases), tissue remodeling (urokinase-type plasminogen activator) and energy production (cytochrome c oxidase), and likely contribute to tumor development in the Eker rat. Importantly, the annexin I gene, implicated in signal transduction, was highly induced at both the mRNA and protein levels in tumor tissue. In addition, toxic tubular dysplasias observed by laser capture microdissection of kidneys from Eker rats treated chronically (10 months) with TGHQ show high mRNA levels of annexin I (6-fold over control). These results suggest that annexin I gene likely plays an important role during the early stages of TGHQ-mediated nephrocarcinogenicity in the Eker rat. (GM 93938, ES 07784).

NBS1 CANCER-SENSITIVITY GENE INFLUENCES GENE EXPRESSION FOLLOWING DNA DAMAGE.
R. P. Arumugam, C. L. Innes, A. N. Heinloth, K. G. Flores, S. O. Sieber, P. R. Bushel and R. S. Pauley. NIEHS Microarray Center, NIEHS, Research Triangle Park, NC and Laboratory of Environmental Carcinogenesis and Management, NIEHS, Research Triangle Park, NC.

The contribution of defects in cellular response to DNA damage and development of cancer, particularly cancer, in patients with genetic susceptibilities is poorly understood. Nijmegen Breakage Syndrome (NBS), a chromosome instability disorder, is associated with hypersensitivity to ionizing radiation and predisposition to cancer. Nibrin, the gene product of NBS1, complexes with Rad50/MRE11 and co-localizes with BRCA1 to regions of damaged DNA, suggesting a role in normal DNA damage recognition and repair processes. We hypothesize that nibrin is important in pathways regulating cell cycle checkpoints in response to DNA damage downstream of, but complementary to, ATM-dependent signaling. We investigated cell cycle checkpoint responses and gene expression changes in mitral diploid fibroblasts in response to ionizing radiation (1.5 to 5 Gy), comparing nibrin-deficient (NBS/-) and wild type (WT) cells. Three assays were used: (i) flow cytometric analysis of BrdU incorporation in DNA synthesis to investigate G1/S checkpoint response; (ii) fluorescence-based DAPI staining to quanitate mitotic cells, a measure of G2/M checkpoint response, and (iii) gene expression profiling, to elucidate molecular pathways involved in checkpoint responses that are defective in NBS. We observe a mitotic delay defect in NBS/- cells following exposure to low doses of gamma irradiation, indicating a role for NBS1 in the G2/M checkpoint. In contrast, at higher doses (5 Gy), NBS/- cells induce a G2/M checkpoint delay. This suggests an important role for nibrin in checkpoint responses and repair at low levels of DNA damage. But at higher levels the activation of compensatory pathways leads to a stronger checkpoint delay. Preliminary comparison of responses to radiation induced damage in WT and NBT cells by microarray analysis, revealed nibrin-dependent transcriptional patterns that may be correlated with defects in cell cycle checkpoints and DNA repair.

APPLICATION OF TOXICOGENOMICS TO DRUG DISCOVERY AND DEVELOPMENT: EFFECT OF A KINASE INHIBITOR ON GENE EXPRESSION IN RAT NASAL TISSUE.

Kinase inhibitors are important therapeutic targets. Because of the large number of intracellular kinases, specificity of a selected kinase inhibitor is crucial for its pharmacological action as well as its potential toxicity. Within a class of kinase inhibitors, toxicity may be similar, but not likely identical. Compound X is a kinase inhibitor that was being developed for treatment of asthma. In a 5-Day oral toxicity study in rats, compound X was shown to produce nasal epithelial toxicity. The aim of this work is two fold: 1) Examine the use of toxicogenomics to evaluate alterations in gene expression in the nasal tissue as a function of treatment with an attempt to elucidate the mechanism(s) of toxicity. 2) Determine the overall utility of toxicogenomics in drug discovery and development. Male rats were dosed with Compound X at 0, 100 and 1000 mg/kg/day for 1 or 5 days. Histopathologic examination confirmed target organs. Toxicogenomic analyses utilized the rat RgU34A chip(Affymetrix). Treatment-related nasal epithelial degeneration and inflammation in the transitional, respiratory and olfactory mucosa was detected in some rats in the high dose group after 1 or 5 days of treatment. Principal component analysis of individual rats showed that the affected rats were clustered separately. Specific genes involved in inflammation, cell proliferation, apoptosis and cell injury were up regulated while genes involved in neuronal transport and signaling, and drug metabolizing enzymes were down-regulated. The alteration in gene expression patterns correlated with treatment-related histopathologic changes.

CHARACTERIZATION OF THE MOLECULAR MECHANISMS UNDERLYING THE HEPATOCARCINOGENESIS INDUCED BY THE NONGENOTOXIC CARCINOGENS, PEROXISOME PROLIFERATORS, IN THE RAT.

Peroxisome proliferators (PP), a diverse group of nongenotoxic compounds, cause cell proliferation, apoptosis suppression and peroxisome proliferation through the activation of the peroxisome proliferator-activated receptor alpha (PPAR-alpha). This cascade of events is believed to contribute to formation of liver tumors in rodents. The aim of this study is to evaluate and characterize the molecular mechanisms of the carcinogenesis induced by these compounds and to determine whether
a specific gene expression pattern could be elucidated in rat liver for the compound class of PP. Compounds included in this study were the hypolipidemic compounds Clofibrate (25 and 250 mg/kg), Fenofibrate (10 and 100 mg/kg), and WY-14643 (25 and 250 mg/kg). They were administered twice a day to Sprague-Dawley rats for up to 5 days. In addition to PP, Phenobarbital (PB, 12 and 120 mg/kg) was also tested in this study as a nongenotoxic hepatocarcinogen acting through a mechanism different from PPAR-alpha activation. Hepatic gene expression profiles were generated using Affymetrix microarrays. Gene expression fold-changes after PP or PB treatment were estimated using a powerful in-house-developed software, GEO2. The transcriptional mapping of the liver was compared with classical toxic end-points such as histopathological evaluation. Preliminary histopathological results indicated a moderate increase in mitotic figures (equivocal of an increase in cell proliferation) and mild centrilobular hypertrophy most likely due to peroxisome proliferation only with the high dose of WY-14643 after a 5 day-treatment. PB induced a mild hypertrophy (proliferation of endoplasmic reticulum) at the highest dose after 5 days. Initial gene expression analysis clearly revealed distinct molecular patterns between PP and PB. These data should provide mechanistic information on early marker genes whose modulation of expression would indicate the carcinogenic potential of PP.

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GP3: GENEPIX POST-PROCESSING SCRIPT FOR AUTOMATED ANALYSIS OF RAW TOXICOGENOMIC MICROARRAY DATA.
E. Dere, M. R. Fielden, R. G. Halgren and T. R. Zacharewski. Department of Biochemistry and Molecular Biology, Institute for Environmental Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.

Toxicogenomic studies generate vast amounts of data that require processing prior to clustering and other analyses. We describe a script to automate the post-processing of raw microarray data captured with GenePix, a widely used commercial microarray image analysis application. The script, written in Perl, filters outlying signal intensities, performs background corrections and normalizes signal intensities between the Cy3 and Cy5 channels. Signal intensities are filtered to ensure they exceed a defined signal threshold of detection while also falling below the saturation level of the microarray scanner. After filtering, valid signals undergo local background correction prior to subsequent normalization. Systematic and experimental biases between the two fluoro-labeled cDNA populations being compared in a two-color fluorescence-based cDNA microarray assay can result in inaccurate quantitation of relative differences in gene expression. To minimize this effect, signal intensities for each fluoro are adjusted to normalize the gene expression distribution in log2 space. By doing so, signal intensities across the entire microarray can be expressed as a percentage thereby minimizing systematic biases in fluorescence incorporation and intensity characteristics and differences in RNA quality and sample handling. The script can operate in batch mode to process data in a high-throughput manner. Other features include abilities to override default parameters of the scripts through a command line interface. Filtered and normalized values are appended to the GenePix results file. In addition, averages and standard deviations of replicate spots are calculated and provided in a summary file. Therefore this script automates the high-throughput post-processing of raw toxicogenomic microarray data generated by GenePix and minimizes human error introduced with repetitive manual adjustments.

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DEVELOPMENT OF A RELATIONAL TOXICOGENOMIC DATABASE FOR THE PREDICTION OF CHEMICAL TOXICITY.
P. C. Boudreau1,2, L. D. Barghouti3,4 and T. R. Zacharewski1,2. Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI; 1Institute for Environmental Toxicology, Michigan State University, East Lansing, MI; 2National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI and 3Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

Toxicogenomic studies generate vast amounts of data that require proper storage in order to facilitate data handling and analysis. The inability to relate changes in global gene expression to specific genes, their function, and other information further complicates data analysis and interpretation. To automate these essential functions, dbZach, a database operating under the Oracle relational database management system has been built. dbZach provides a core database that handles all aspects of our multi-species toxicogenomic/microarray investigations including: 1) storage of important cDNA/EST clone information, such as sequence, 2) frequent, automated updates of gene annotation information, 3) microarray data storage, 4) physical mapping data for each microarray utilized and constructed within the Lab. Currently, dbZach is comprised of four main subsystems: 1) the Clones Subsystem, 2) the Microarray Subsystem, 3) the Gene Function Subsystem, and 4) the Lookup Subsystem. The subsystems interrelate to find data within a dataset, and define relationships between tables. Querying dbZach for information regarding mT2Z0.6, our mouse microarray, reveals the availability of 2, 288 cDNA/ESTs representing 1, 948 unique genes. Ninety-five percent of the available cDNA/ESTs can be assigned to a specific cluster with the average cluster represented by 1.25 clones. Less than 1% of the genes on our marine microarray, mT2Z2.6, are represented by more than 3 clones. As part of our effort to develop a transcript profile database predictive of chemical toxicity, future planned developments for dbZach include compliance with MIAME standards for microarray experiments, statistical analysis functionality, and comparisons of global gene expression data stored within dbZach.

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UTILIZATION OF SUBTRACTIVE HYBRIDIZATION AND MACROARRAYS TO DETERMINE ALTERED GENE EXPRESSION IN PYRENE EXPOSED MUMMICHOG (FUNDULUS HETEROCICLUS).
J. A. Roling, N. L. Maples, L. J. Bain and W. S. Baldwin. Biological Sciences, University of Texas at El Paso, El Paso, TX.

Mummichogs are ideal for monitoring the sublethal effects of pollutants in estuarine environments along the coast because of their small home range. Estuaries, due to their proximity to humans are susceptible to anthropogenic stress from numerous sources, such as polycyclic aromatic hydrocarbons (PAHs), pyrene. Therefore, mummichogs were exposed in the laboratory to several concentrations of pyrene at 0, 10, 20, 35, and 50 ppb in 7-day static renewal exposures and alterations in gene expressions in liver were examined. Previous work in our laboratory has demonstrated that pyrene induces XR repressor transposon and P450 1A RNA levels. Subtractive hybridization was performed on mRNA from the pyrene exposed mummichog to enhance the production of differentially expressed transcripts and to find novel biomarkers of PAH exposure in mummichog. The transcripts were cloned and 96-gene macroarrays were produced. Control and treated mummichog liver cDNA was then incubated onto the blots and changes in gene expression were measured. Genes demonstrating differential expression due to pyrene will be sequenced and presented at SOI.

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DEVELOPMENT OF MICROARRAYS AS A TOOL FOR DISCOVERING ENVIRONMENTAL EXPOSURE INDICATORS.

Toxicogenomics is research that can be applied to identification of differential gene expression in wildlife and predict effects of environmental stressors. We are developing a multiple stressor exposure model using fathead minnow (Pimephales promelas) cDNAs in a microarray platform. A two-pronged approach has been developed to examine specific gene expression and to identify patterns over a portion of the expressed genome through the use of subtractive libraries. In order to start synthesis of a fathead minnow specific gene chip for array analysis, homologs of specific genes were identified in P. promelas using degenerate PCR primers based on conserved homology across mammalian and non-mammalian taxa. Categories of genes include DNA repair genes, receptor and non-receptor kinase genes, transcription factors, and genes involved in apoptotic cascades such as caspases. Initially, single exposure-specific microarrays were employed using subtractive cDNA clones to identify gene patterns for single-exposure analysis. Multiple exposures can also be assessed at the microarray level to gain a better understanding of relative bioavailability of environmental stressors present in mixtures. Thus, the use of microarray technology to examine gene expression is a powerful tool in understanding the impacts of environmental exposures.

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NCBI-BASED ANNOTATION AND CROSS-COMPARISON OF MICROARRAY CHIP FEATURES.
W. B. Matter1, S. D. Penri1, R. D. Tyler1, W. E. Pennie1 and D. E. Robinson1. Investigative Toxicology, Pharmacia, Kalamazoo, MI; 2Health and Environmental Sciences Institute, ILSI, Washington, DC; 3Investigative Toxicology, Syngenta, Macclesfield, United Kingdom and 4Safety Assessment, GlassSmithKline, Research Triangle Park, NC.

Transcript profiling (TnP) uses genomic technologies such as microarrays to measure the response of hundreds to thousands of transcripts to various environmental perturbations. These studies are carried out to determine not only the response of certain genes, but also overall patterns of gene expression. A toxicological mechanism may be inferred from the response of a particular transcript, or from the pro-
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STUDIES ON THE MECHANISM OF ACTIVATION OF 7H-BENZ[A]FLUORENE.

B. Parmo1, E. H. Weyand1, I. G. Goldstein1, J. Q. Wang2 and R. G. Harvey1
1. Pharmaceutical Chemistry, Rutgers University, Piscataway, NJ; 2. Environmental Sciences, Affymetrix/Omni 3.0 Rat Genome GeneChip®. 1986 are shared in common with the 5902 “genes” on the Incyte RatGen 3 microarray. Likewise, the U34 GeneChip has 232 “genes” in common with Phase-1 Toxicology’s 250 “gene” microarray. While cross-referencing elements based on accession number alone is not precise, this system does provide a rapid method for determining some similarities and a means for updating annotation on a regular basis.

1486
"P"-POSTLABELING OF PHOTOCHEMICALLY BOUND DNA ADDUCTS.

A. M. Jeffrey1, J. D. Duan1, D. Scide1, H. G. Erzenmann2, E. von Keutz2 and G. M. Williams1
1. Dept. Pathology, New York Medical College, Valhalla, NY and 2. Institute of Toxicology, Bayer AG, Wuppertal, Germany.

Many compounds can be photocarcinogenic to genotoxic products. We have investigated three photocarcinogenic compounds by "P"-postlabeling for DNA adducts: BAY y3118, an experimental fluorouracil analogue; chlorpromazine (CPZ) an antiemetic and antipsychotic, and 5, 6'-dihydroxy-2-benzpyrene (DHPyP) a compound related to 3,4-benzpyrene used in the PUVA treatment of psoriasis. BAY y3118 has not been investigated for DNA adduct formation, although it is photocarcinogenic (Arch Toxicol., 74, 555, 2000), while CPZ is known to form DNA adducts in vitro (Chem Biol Interact., 51, 273, 1984) and this is also true of DHPyP. BAY y3118 and CPZ were incubated in HeLa cell cultures (Carcinogenesis, 15, 89, 1994) in the presence of UV light. All three compounds were photocarcinogenic by 345 nm UVA light in the presence of calf thymus DNA and the DNA purified by ethanolic precipitation. Photocarcinization of BAY y3118 yielded DNA that exhibited a substantial fluorescence (Ex 350 nm, Em 450 nm). This modification did not occur when BAY y3118 was irradiated prior to mixing with the DNA using the "P"-postlabeling method. When the same method was used, a short-lived species during the photochemical reaction. Using UV-photocarcinization of BAY y3118, photocarcinization in radioactive binding to DNA corresponding to about 70 DNA adducts in 10' normal nucleosomes. Oxygen was not required since, under anaerobic conditions, the fluorescence associated with the purified DNA was even higher. Postlabeling of these DNA samples either directly, using unlabeled P, or column (Water Oasis HLB) enrichment of the samples showed DNA adducts were formed with CPZ but not with the other two chemicals. TMP, however, was positive by the specific approach developed for it that involves digestion to di-2-deoxyribonucleoside monophosphates (Carcinogenesis, 15, 89, 1994), but even using these conditions, BAY y3118 was negative. Thus, although successful with CPZ and TMP, we have not detected DNA adducts by "P"-postlabeling with BAY y3118. (Supported by NCI Grant R01-CA60696)

1487
APURINIC/APYRIMIDINIC (AP) SITES, INTERMEDIATES IN DNA REPAIR, ARE NOT INCREASED BY ETHYLENE OXIDE INHABITATION IN RATS.

S. Asakura, J. Nakamura and J.A. Swenberg, Environmental Sciences and Engineering, UNC-Chapel Hill, Chapel Hill, NC.

Ethylene oxide (EO) is an important industrial chemical and gaseous pollutant that is mutagenic and carcinogenic in rodents. 7,8-Dihydroxy-8-oxo-2'-deoxyguanosine (8-oxo-dG) represents 90% of the DNA adducts induced by EO. While not promutagenic itself, HEG can cause mutations through the formation of apurinic (AP) sites. We developed a sensitive assay to detect AP sites in genomic DNA, which can quantitate <1 AP site/10 nucleotides. A newly established AP site cleavage assay which was used for in vivo studies, was charac-

1488
ESTROGENIC PHENOL AND CATECHOL METABOLITES OF PCBs DOWN REGULATE CATECHOL-O-METHYLTRANSFERASE.

C. E. Garner1,2, F. Zhe-lui1, D. Ramsden1, H. B. Mathews1, L. T. Burkett1 and S. L. Ho1
1. DSER, Schering-Plough Research Institute, Lajette, NJ; 2. Lethal of Pharmacology and Chemistry, NIEHS, Research Triangle Park, NC.

PCB mixtures induce liver tumors in female rats and this effect may be due to altered estrogen metabolism. This laboratory has demonstrated that metabolism of PCBs is potent inhibitors of COMT and may contribute to reduced clearance of genotoxic catechol estrogens (CEs). Liver S9 of female SD rats treated with carcinogenic doses of Aroclor 1254 for 21 days (10 mg/kg/day) showed ca. 60% reduction of COMT activity towards CEs. We hypothesize that estrogenic PCB metabolites may contribute to reduced COMT activity via the estrogen receptor, which has been demonstrated to regulate COMT. Test estrogens, 2, 4, 6-trichloro-4- biphenyl and 2, 4, 6-trichloro-3, 4-biphenyl were incubated in human MCF-7 cells expressing the ER and COMT for 48 hours and expression of COMT determined. Western blot analysis showed that COMT protein levels were reduced in a concentration dependent manner by both metabolites. Additionally RT-PCR analysis showed a concentration dependent reduction of COMT RNA suggesting that the PCB metabolites reduced COMT expression at the transcriptional level. The above suggests that COMT levels may be reduced via interaction between PCB metabolites and the ER. The above information, coupled with our prior work, supports the hypothesis that PCB metabolites may contribute to PCB-mediated.

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carcinogenesis through reduced clearance of endogenous and xenobiotic catechols by reducing both catechol O-methyltransferase levels and activities.

1489
FUNCTIONAL CHARACTERIZATION OF N-ACETYLTRANSFERASE 1 (NAT1) AND 2 (NAT2) IN MAMMARY PRIMARY CELL CULTURES FROM RAPID AND SLOW ACETYLATOR SYRIAN HAMSTER CONGENIC AT NAT2.

G. H. Xiao, P. A. Jefferson and D. W. Hein. *Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Aromatic and heterocyclic amine carcinogens present in the diet and in cigarette smoke induce breast tumors in rodents. N-acetyltransferase-1 (NAT1) and N-acetyltransferase-2 (NAT2) are important in the metabolism of these carcinogens. Human epidemiological studies suggest that genetic polymorphisms in NAT1 and/or NAT2 predispose to breast cancer in women exposed to these carcinogens. We hypothesized that NAT1- and NAT2-catalyzed activation and/or deactivation of these carcinogens within the breast may affect breast cancer risk. Our laboratory constructed a rapid and slow acetylator Syrian hamster model congenic at the NAT2 locus that we used to investigate NAT1 and NAT2 expression in mammary primary cell cultures. p-Aminobenzoic acid (PABA), and 4-aminobiphenyl N-acetyltransferase and N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine O-acetyltransferase activities were (p<0.05) higher in mammary cell cultures from rapid than in slow acetylators. Sulfamethazine (an NAT1-specific substrate) N-acetyltransferase activity was low and did not differ between rapid and slow acetylators. NAT1 and NAT2-specific mRNA were quantitated by real-time polymerase chain reaction. Levels of NAT1 and NAT2 mRNA were similar, but NAT2 mRNA was higher in rapid than in slow acetylators. Hamster mammary cells cultured in the medium supplemented with PABA for 14 days showed a significant decrease (50-70%) in NAT2 activity. The loss of activity was concentration-dependent (EC50 4 μmol/L) and selective because PABA treatment showed no effect on NAT1 activity. The loss of NAT2 activity was associated with reduction in the amount of NAT2 but not NAT1 mRNA. These studies clearly show that NAT1- and NAT2-catalyzed acetylation of aromatic and heterocyclic amine carcinogens in mammary cell cultures modified by substrate regulation of NAT2 expression. Partially supported by USPHS grant CA-34627 from the National Cancer Institute.

1490
N-ACETYLTRANSFERASE 2 PHENOTYPE IN PAINTERS WITH UROTHELIAL BLADDER CANCER AND NON-DISEASED COLLEAGUES.

K. Golkas, W. Weisenthöfer, P. Jedrasik, F. Geller, M. Blaszkiewicz and H. M. Bolt. *Institute of Occupational Physiology at the University of Dortmund, Dortmund, Germany.*

Aim: This study was designed to evaluate the impact of N-acetyltransferase 2 (NAT2, substrate aromatic amines) on bladder cancer in painters. Background: Until the beginning of the 60s, painters in Germany have used, among others, azo dyes based on carcinogenic aromatic amines. Materials and Methods: Sixteen painters, from different areas in Germany suffering from transitional cell carcinoma of the bladder, and 26 healthy painters from the same areas and at the same age (±5 years) as controls, were investigated. All painters were phenotyped for NAT2 by the molar ratio of two caffeine metabolites in urine, determined by a HPLC method. All painters were asked for the period of work as a painter and for their life-time smoking habits. Results: Exposure against paints (colorants) started in 1950 in cases (SD 9.3) and controls (SD 9.6). Fourteen cases and 23 controls had been exposed to paints before 1960. Age at first exposure against paints was 15.5 years (SD 5.3) in cases and 16.3 years (SD 4.9) in controls. Cases had worked 31.1 years (SD 15.0), and controls had worked 44.8 years (SD 7.2) as a painter. Four cases and 7 controls were non-smokers. In the present study, 88% of 16 diseased painters and 65% of 26 non-diseased painters were of the slow acetylation phenotype. Conclusions: The results suggest that the "slow" acetylation status is an individual risk factor for bladder cancer in persons occupationally exposed to amounts of carcinogenic aromatic amines released from water-soluble azo dyes.

1490A
N-ACETYLTRANSFERASE 1 AND 2 GENOTYPES AND PROSTATE CANCER.


N-acetyltransferase-1 (NAT1) and N-acetyltransferase-2 (NAT2) are important in the bioactivation and deactivation of aromatic and heterocyclic amine carcinogens that induce prostate tumors in the rat. We investigated the association of genetic polymorphisms in NAT1 and NAT2, alone and in combination, with human prostate cancer. Incident prostate cancer cases and controls in a hospital-based case-control study were frequency-matched for age, race, and referral pattern. Slow acetylator NAT2 genotype exhibited borderline association with prostate cancer (OR, 1.75; 95% CI, 0.86-3.54), but the association was greater for individuals homozygous for NAT2 alleles (NAT2*2) with lowest NAT2 catalytic activity (OR, 2.43; 95% CI, 0.96-6.12; P = 0.08). In contrast, individuals possessing the putative rapid acetylator NAT1 genotype (one or more NAT1*10 alleles) were over-represented in prostate cancer (OR, 2.17; 95% CI, 1.08-4.33; P = 0.03). Combinations of NAT1*10 with NAT2 slow genotypes (OR, 5.08; 95% CI, 1.56-16.5; P = 0.008) or with NAT2 very slow acetylator (homozygous NAT2*2) genotype (OR, 7.50; 95% CI, 1.55-15.4; P = 0.016) further increased prostate cancer risk. The results of this pilot study suggest increased susceptibility to prostate cancer in combinations of NAT1*10 and the slowest NAT2 genotypes, but needs to be investigated further in larger cohorts and in other ethnic populations. Partially supported by USPHS grant CA-34627 from the National Cancer Institute.

1491
THE PRESENCE OF DIMETHYLSERINIC ACID (DMA) IN THE URINE OF RATS TREATED WITH DIMETHYLSERINIC ACID (DMA).


DMA is carcinogenic to the rat urinary bladder when administered in the diet or drinking water. At a dietary dose of 100 ppm, it produces cytotoxicity within six hours and increased proliferation (hyperplasia) by seven days of administration. 2,3-Dimercaptopropionic acid (DMPS) reacts with trivalent forms of arsenic and has been used in the treatment of arsenic poisoning. When DMPS is co-administered with DMA, the cytotoxicity and proliferative tendency of TNM0 and the slowest NAT2 genotypes, but needs to be investigated further in larger cohorts and in other ethnic populations. Partially supported by USPHS grant CA-34627 from the National Cancer Institute.

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PREDICTION OF THE CARCINOGENESIS OF FUMONISIN B, IN MALE AND FEMALE F344/NINCTR RATS FROM URINARY AND TISSUE SPHINGOLID CHANGES.


Fumonisin B1 (FB1) is a mycotoxin that has been detected world-wide as a result of growth of Fusarium species in corn. FB1 was carcinogenic in a two-year feeding study inducing renal tubule tumors in male F344/NINCTR rats at 50 and 150 ppm, but was not carcinogenic at the highest dose (100 ppm) in females. FB1 inhibits de novo sphingolipid synthesis through inhibition of ceramide synthase, resulting in increased tissue levels of sphingolipid (Sa). This increase is likely to be playing a role in a biomarker of exposure. Urinary sphingolipid were determined in the feeding study after 6, 10, 14, and 26 weeks, and tissue levels of sphingolipid were determined at the interim sacrifices and in some cases at 2-years. Urinary Sa was increased in the males at 15, 50, and 150 ppm, and in the females at 50 and 100 ppm. Urine Sa/sphingolipid (Sa/S0) ratios were likewise consistently increased in the males at 15, 50, and 150 ppm, and in the females at 50 and 100 ppm. Kidney Sa and Sa/S0 were elevated in the males at 15, 50, and 150 ppm, and in the females consistently only at 50 and 100 ppm. Liver Sa/S0 ratios were only elevated at the interim sacrifices in the female rats at 100 ppm. These results demonstrate that induction of tumors in the kidney but not the tumor induction in the male rat correlates with Sa and Sa/S0 ratios in these tissues. These results also show a correlation between the renal tubule carcinogenicity of FB1 and the extent of accumulation of Sa in the two rat urine and kidney: however, since Sa levels were also increased in the female urine and kidneys in the absence of carcinogenicity, the Sa biomarker may not be predictive of tumorigenic outcome in the kidney.

THE EFFECT OF ETHANOL ON THE PROLIFERATION OF BOTH ESTROGEN SENSITIVE LEYDIG CELLS AND ESTROGEN RECEPTOR POSITIVE MCF-7 CELLS.

J. W. DuMond, A. Sharga and D. Roy. EHS, UAB, Marbury, AL.

Excess alcohol consumption has been identified as a risk factor for breast and other hormonal cancers. Now alcohol is considered as a co-carcinogen. The mechanism for increased hormonal cancers is not known. Recently it has been reported that ethanol induces proliferation of MCF-7 cells and increases estrogen receptor expression. While the mechanism for these effects are not known, they seem to support epidemiological evidence that link alcohol consumption with an increased risk of breast cancer. In this study we have examined the influence of low and high concentrations of ethanol on testicular Leydig cells and MCF-7 cells. We examined the effect of ten different concentrations of ethanol on the proliferation of TM3 Leydig and MCF7 cells. We report for the first time that exposure of cells to a low dose of ethanol (1 mM) produce significant increase in cell growth, and the doses of ethanol greater than 5 mM produced inhibition of cell growth. Exposure of Leydig cells to ethanol in combination with tamoxifen failed to significantly lower the ethanol-mediated proliferation. It appears that alcohol may be playing a role in human hormonal carcinogenesis presumably through influencing signaling pathways involved in the regulation of cell cycle.

INHIBITION OF ESTROGEN RECEPTOR NEGATIVE BREAST CANCER CELL GROWTH BY SELECTIVE ARY HYDROCARBON RECEPTOR MODULATORS.

L. Korba, A. McDougal and S. H. Sadegh. Veterinary Physiology & Pharmacology, Texas A&M University College Station, TX.

Aryl hydrocarbon receptor (AhR) agonists induce inhibitory AhR-estrogen receptor alpha (ERa) crossstalk in breast cancer which is accompanied by inhibition of cell cycle progression in vitro and tumor growth in rodent models. Growth of ER-negative MDA-MB-468 breast cancer cells is also inhibited by AhR agonists and in Balb/c athymic nude mice bearing MDA-MB-468 cell xenografts, the selective AhR modulators (SRHMs) 6-methyl-1,3, 8-trichlorodibenzo[alpha]furan (MCDF) and dimethyl-methane (DIM) inhibited mammary tumor growth. We have further investigated the Ah responsiveness of several other ER-negative breast cancer cell lines and the growth inhibitory effects of MCDF, DIM and related SRHMs. Cell lines were initially screened for induction of CYPII probe (EROD) activity after treatment with 10 nM TCCD for 24 hr. The results showed that EROD activity was significant induced by TCCD in HCC-38, MDA-MB-453, MDA-MB-157, and MDA-MB-435 cells, whereas no induction was observed in BT-20 cells. In parallel studies, the effects of TCCD, MCDF and DIM on proliferation of the four ER-negative breast cancer cell lines were also investigated. TCCD and the SRHMs inhibited growth of the four ER-negative breast cancer cell lines. For example, the IC50 values for inhibition of MDA-MB-157 cell growth using 2.5% serum were ≤ 1 nM TCCD, 100-1000 nM DIM and 10-100 nM MCDF. The IC50 values for these compounds varied among the cell lines and was also dependent on percent serum used. Current studies are focused on the AhR-mediated mechanisms of cell growth inhibition. (Supported by NIH CAG65081, ES04176 and ES060216)

EFFECT OF CHRONIC HEXACHLOREZOBENZONE (HCB) EXPOSURE ON TELOMERASE ACTIVITY IN HUMAN MAMMARY EPITHELIAL CELLS.

R. Audet, M. A. Poirier, G. Lassonde and M. Charbonneau, Human Health Research Centre, INRS-Institut Armand-Frappier, Université du Québec, Montréal, PQ, Canada.

HCB is a widespread environmental contaminant virtually present in 100% of North American women. Epidemiological studies suggested that a higher HCB level could be a risk factor for breast cancer. HCB has been shown to increase the phosphorylation of the EGF receptor family, such as EGF, in human mammary epithelial cells. Such proinflammatory alterations can promote the appearance of cells with a proangiogenic phenotype. Normally absent in somatic cells, telomerase is induced in most tumour cells enabling them to divide indefinitely and become immortal. In order to evaluate the potential of HCB to cause epigenetic changes akin to a proangiogenic phenotype, cultures of a non-tumorigenic human mammary epithelial cell line, MCF10-A, were chronically exposed to 20 μM HCB (0.1% in DMSO) or to DMSO alone. Cells were grown in DMEM supplemented with 20 ng/ml EGF, 10 mg/ml insulin, 5% horse serum, and telomerase activity was measured using the telomeric repeat amplification protocol at specific passages. Cell proliferation was assessed throughout the experiment by calculating the population doubling time. Following approximately 25 passages of continuous exposure to HCB, a marked increase in telomerase activity was observed; these cells were proliferating at a 2-fold higher rate. When these cultures were subsequently maintained with DMSO alone, telomerase activity remained higher than in the continuously DMSO-treated cells; the increased cell proliferation also remained higher. The latter observations imply that HCB-induced epigenetic changes are long lasting. Increased telomerase activity is a very widespread phenotype of tumorigenic cells. Overall, results show that a major mechanism enabling mammary epithelial cells to become immortal is activated by continuous HCB exposure. This work supports the contention that environmental contaminants can be a risk factor in breast cancer development. (Supported by the Toxic Substances Research Initiative and the Pirm of Research in Environment Etiology of Cancer).

EFFECTS OF ORGANOCHLORINES ON c-erb-B-2 (new) SIGNALLING PATHWAY IN HUMAN MAMMARY EPITHELIAL CELLS.

S. Girard, M. A. Poirier and M. Charbonneau, Human Health Research Center, INRS-Institut Armand-Frappier, Université du Québec, Montréal, PQ, Canada.

Environmental factors such as chemical exposure to contaminants, dietary habits and lifestyle could have an important role in breast cancer development (BCD). Organochlorines are lipophilic contaminants distributed in breast tissue, however their role in BCD is debated. New is an oncogene protein belonging to the epidermal growth factor receptor (EGFR) family which correlates with poor prognosis for breast cancer patients. In human mammary epithelial cells organochlorines could perturb receptor signaling pathways, such as that linked to the new receptor overexpressed in several breast cancers. This research aims to assess the in vitro effect of an environmental mixture (Mix), mainly correlates with 3 PCBs, 7 polychlorinated dibenzo-p-dioxins (PCDDs) and 6 polychlorinated dibenzofurans (PCDFs) and found in human breast milk, on constitutive and phosphorylated levels in a non tumorigenic human mammary epithelial cell line (MCF-10A). Cells, grown in DMEM medium supplemented with 20 ng/mL EGF, 10 μg/mL insulin, 5% horse serum, were exposed to the Mix for 0, 4, and 30 min, then were collected with lysis buffer for Western Blot analysis: expression (erb-B-2/HER-2 antibody) and activation (phospho-erb-B-2/HER-2 antibody) of new were measured. Data were expressed as the ratio between phospho-new over new using actin levels for normalization. Mix caused a 2- to 4-fold increase of the phospho-new/new ratio at both time points. In another series of experiments a long term exposure (more than 30 days) with hexachlorobenzene (HCB), an important environmental contaminant, also caused a 2-fold increase in the ratio. Overall, these observations show that environmental contaminants cause an overactivation of a key tyrosine kinase receptor frequently overexpressed in breast cancer tissue. Downstream events may involve overactivation of proteins, such as Akt and IκB. This work brings biological plausibility to the involvement of environmental contaminants in BCD. (Supported by the Toxic Substances Research Initiative, Health Canada)
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**EFFECT OF 2',4', 4', 5, 5'-HEXACHLOROBIPHENYL (PCB-153) ON HEPATOCYTE PROLIFERATION IN MICE DEFFICIENT IN THE P50 SUBUNIT OF NF-kB.**


Polychlorinated biphenyls (PCBs) are a group of synthetic chemicals that induce and promote liver tumors in rodents. Our previous studies showed activation of the hepatic transcription factor NF-kB in rats treated with PCBs and an increase in hepatocyte proliferation that is correlated with the NF-kB activation. In this study, the effect of NF-kB activation on hepatocyte proliferation after PCB administration was analyzed in male B6129 mice and mice deficient in the p50 subunit of NF-kB. In a 2-day study, mice received a single i.p. injection of corn oil or PCB-153 (300 µmol/kg) and were euthanized after 48 hours. In a 21-day study, mice received six i.p. injections of corn oil or PCB-153 (100 µmol/kg) and were euthanized 4 days after last injection. Nuclear NF-kB DNA binding activity, measured by electrophoretic mobility shift assay, was present in control wild type mice and increased with PCB-153 in the 2-day study. NF-kB DNA binding activity was less in the p50-/- mice and did not increase with PCB-153. Hepatocyte proliferation was measured in mice with the 5-bromo-2'-deoxyuridine (BrDU) labeling index. Control wild type mice had the same level of cell proliferation as the control p50-/- mice. Cell proliferation increased in the wild type mice treated with PCB-153 in both studies. PCB-153 did not change the cell proliferation in the p50-/- mice in the 2-day study, but the p50-/- mice had a slight increase in cell proliferation after 21 days. These data indicate that NF-kB is involved in the proliferative changes in response to PCB-153. (Supported by S2 ES07380)

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**EFFECTS OF VITAMIN E ON NF-kB DOWNSTREAM EVENTS IN P50-/- MICE TREATED WITH THE PEROXISOME PROLIFERATOR, CIPROFIBRATE.**


Peroxisome proliferators (PPs) are a diverse class of chemicals, including some hypolipidemic drugs and plasticizers, which cause a dramatic increase in the size and number of hepatic peroxisomes in rodents and eventually lead to hepatic tumors. The pathway to tumorigenesis by PPs has not been delineated, but oxidative stress is believed to alter signal transduction pathways thereby leading to cell growth disturbances. Nuclear factor-kB (NF-kB) is a transcription factor activated by reactive oxygen and is involved in cell proliferation and apoptosis. We previously found that the peroxisome proliferator ciprofibrate (CIP) activates NF-kB and that dietary vitamin E is effective in decreasing CIP-induced NF-kB DNA binding. We hypothesized that increased dietary vitamin E reduces CIP-induced oxidative stress and cell proliferation by reducing the activation of NF-kB in mice. Sixteen B6129 female mice and twenty homozygous NF-kB deficient mice (p50-/-) were fed a purified diet containing 10 or 20 ppm vitamin E (tocoherol acetate) for 28 days. At that time, half of the wild-type and half of the p50-/- mice were placed on the same diet with 0.01% CIP for 10 days. Nuclear extracts were prepared from the livers and NF-kB DNA binding activity was determined using electrophoretic mobility shift assays. Hepatocyte proliferation was determined immunohistochemically by measuring the 5-bromo-2'-deoxyuridine (BrDU) labeling index. In the wild-type mice, CIP-treatment increased the p65:50 NF-kB activity and increased cell proliferation. Neither endpoint was significantly altered with dietary vitamin E levels. In contrast, the p50-/- mice had lower NF-kB activity and higher basal levels of cell proliferation and lower GSH/GSSG. The p50-/- mice fed higher dietary vitamin E and treated with CIP had 60% lower cell proliferation in comparison to the group fed low vitamin E. Thus, the effects of vitamin E on cell growth parameters are not solely through decreased NF-kB activation.

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**EFFECT OF KUPFFER CELL ACTIVATION ON LIVER FOCAL LESION GROWTH.**

P. J. Klein, L. M. Kamenulis and J. E. Klausing. Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

The induction of hepatocellular neoplasia in rodents is a multiphase process involving a series of cellular modifications. While most studies have focused on the hepatocyte as the target cell, recently a role for other liver cell types, specifically the Kupffer cell, have been suggested to participate in the carcinogenesis process. The purpose of the present study was to investigate the effect of Kupffer cell activation on preneoplastic focal lesion growth in liver. Hepatocellular foci were induced in F344 male rats using diethylnitrosamine. Following foci lesion formation, selected rats were treated with bacterial lipopolysaccharide (0.25 mg/kg i.p. weekly). Rats were sampled after 7 and 28 days of treatment, and livers were examined for DNA synthesis, apoptosis, and mitosis in normal and focal liver lesions. In addition, the relative area and number of hepatic foci lesions was quantified using stereologic methods in H and E and glutathione S-transferase (GST-P) stained histologic sections. Lipopolysaccharide treatment caused a significant increase in GST-P positively stained foci area as compared to control. In addition, a 2-fold increase in relative focal lesion volume (GST-P and H and E) were seen in lipopolysaccharide treated rat liver compared to control. This increase appeared to be mainly localized to eosinophilic foci. While lipopolysaccharide produced an increase in focal lesion size no increase in number of Kupffer foci was seen. Lipopolysaccharide treatment also produced an increase in DNA synthesis in both normal liver and focal lesions, although the increase was markedly greater in focal hepatocytes. From these results, it appears that Kuppfer cell activation by lipopolysaccharide functions at the promotion stage of the multistage carcinogenesis process.

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**MECHANISMS OF BUTYROXETHANOL CARCINOGENESIS IN THE RAT AND MOUSE.**

A. M. Slezko, L. M. Kamenulis, Y. Xu and J. E. Klausing. Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

Butyroxethanol induces an increase in liver tumors (hemangiosarcomas) following chronic treatment in the mouse but not in the rat. Concomitant with tumor development is the induction of hemolysis and an increase in hemosiderin deposition in the liver. Previous studies by our group have shown an increase in oxidative damage in mouse liver following butyroxethanol treatment. These observations have led to our proposal that butyroxethanol induces tumors indirectly through activation of Kupffer cells (via free loading) that in turn produces reactive oxygen species as well as the release of cytokines resulting in induction of cell proliferation in the target cells. The present studies examined whether butyroxethanol induced oxidative stress and altered cell growth in the susceptible species (B6C3Fl mouse) and in the non-susceptible species (F344 rat) following treatment with 0, 225, 450 and 900 mg/kg butyroxethanol for 7, 14, 28, and 90 days. Butyroxethanol produced a biphasic response in the mouse. Butyroxethanol (450 and 900 mg/kg) resulted in an increase in oxidative damage in DNA (OH8IDG) and lipid (malonaldehyde) at both the 7 and 90 treatment points. Hepatic vitamin E levels were also decreased at these time points. DNA synthesis was increased in liver endothelial cells at 7 days while DNA synthesis in hepatocytes was only increased at 90 days. No increases in oxidative damage or DNA synthesis were seen in the rat at any dose or time point examined. No changes in apoptosis or mitosis were observed in the liver of either species at any dose or time point. In summary butyroxethanol caused an increase in oxidative damage and DNA synthesis in the mouse (susceptible species) in cell types that correspond to the observed tumors, while no changes were seen in the rat (non-susceptible species). These results support our proposal that butyroxethanol-induced neoplasia occurs through the induction of oxidative stress resulting in an increase in DNA synthesis in the target cell types in the mouse liver.

1501

**MECHANISMS OF MACROPHAGE ACTIVATION BY PEROXISOME PROLIFERATORS.**

O. M. Smutny, X. Li, M. D. Wheeler and R. G. Thurman. Curriculum in Toxicology, Department of Pharmacology, Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Although the peroxisome proliferator receptor (PPAR), localized exclusively in hepatocytes, is required for peroxisome proliferator-induced cell proliferation, evidence has accumulated that Kupffer cell-derived oxidants and nitrogentic cytokines are also required in this mechanism. However, it is unknown how peroxisome proliferators activate Kupffer cells. Since circulating WY-14, 643 is almost exclusively bound to albumin in sires, it was hypothesized that WY-14, 643 is recognized by macrophage scavenger receptors which function physiologically to recognize modified lipoproteins and albumin. Indeed, the data presented here show that WY-14, 643 bound to albumin leads to a significant but transient increase in intracellular calcium in a murine macrophage cell line (RAW 264.7 cells); this effect was nearly completely prevented by preincubation with the scavenger receptor inhibitor polyinosinic acid. Interestingly, WY-14, 643 also lead to a rapid increase in ERK1/2 expression suggesting a role for the MAP kinase pathway in addition to calcium-dependent signaling in mediating an inductive response to peroxisome proliferators. Collectively, these data begin to address for the first time how peroxisome proliferators initially activate Kupffer cells to ultimately result in oxidant and nitrogentic cytokine production.
and promotion, and the embryo has limited protective antioxidative mechanisms, leaving it at potentially high risk. We have previously shown that maternal supplementation with the antioxidant vitamin E (VE) alters levels of endogenous embryonic DNA oxidation. Here, to determine whether cancer can have in utero origins, we examined the effect of maternal dietary VE (dl-tocopherol-acetate) supplementation on spontaneous postnatal tumorigenesis, using high-dose VE and the cancer-prone F344 rat and mouse model. Virgin heterozygous (+/-) p53-deficient females were placed on either a normal or 10% [w/w] VE-supplemented diet for 4 weeks and mated with +/- p53-deficient males. VE supplementation was stopped on the day of birth. The offspring were p53-genotyped and observed for spontaneous tumor development. Compared to offspring from dams supplemented with control diet, in utero exposure to VE enhanced spontaneous tumor formation in both +/- and -/- p53-deficient offspring. At 40 weeks of age, VE-exposed +/- p53 pups had less than half the survival rate of +/- p53 control offspring (22% vs. 49%) (p<0.02). Among +/- p53 offspring, 78% of the VE offspring were still alive at 14 weeks of age, compared to 92% in the control group (p<0.04). This tumorigenic enhancement is consistent with a study in CD-1 mice in which high-dose VE failed to reduce endogenous fetal DNA oxidation, and shows that high-dose VE can exacerbate rather than block some ROS-mediated pathways. These preliminary results indicate that some cancers originate in utero and can be substantially modified by altering embryonic redox status, suggesting novel carcinogenic mechanisms and potential therapeutic strategies. [Support: NCI, CHIR]

1503 PUTATIVE COLON CANCER RISK FACTORS SPECIFICALLY INDUCE DAMAGE OF P53 IN PRIMARY HUMAN COLON CELLS.


Introduction: Colon cancers are strongly linked to dietary factors. Although it has been possible to identify whole foods as increasing risks it awaits clarification which individual compounds are involved in the molecular level. One group of candidates includes α, β-unaturated aldehydes (products of lipid peroxidation) and reactive oxygen species (result from oxidative stress). Both are elevated in tissues and body fluids during unhealthy diets. Also it is of high interest to experimentally assess their potency for inducing cancer-related endpoints in colon cells, and thus to obtain more evidence for their relative impacts during colon carcinogenesis. For this we have developed a novel application utilizing fluorescence in situ hybridization of isoalcohol ("correct" structures, single cell microelectrophoresis) with p53 specific probes (CorrectFish).

Methods: Human epithelial colon cells were isolated from surgical tissue by enzyme digestion. Test compounds were trans-2-hexenal (600-1600μM) and H2O2 (18.75-150μM). Global DNA damage was determined with the alkaline comet assay and specific breakage of p53 with CorrectFish. Results: Both compounds induced DNA damage in primary human colon cells. The highest doses induced similar degrees of damage with similar staining patterns for p53 migration. Conclusion: The results clearly show that trans-2-hexenal and H2O2 induce genetic damage in primary human colon cells. Moreover, for the first time it has been shown that they induce specific genetic damage in p53.

The novel and relevant method of CorrectFish now allow being developed to compare p53 breakage with damage in APC and K-Ras. The approach of measuring genotoxic impact in the cancer relevant genes of human colon cells offers new unique possibilities to study potential risk factors and learn more on the etiology of colon cancer.

1504 MODULATION OF BENZ(A)PYRENE-INDUCED P53 BY ACROLEIN.

S. S. Biswal*, T. Maxwell and J. P. Kehrer, Dept. of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX and Dept. of Toxicology Science, Department Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins Univ., Baltimore, MD.

Acrolein, a highly electrophilic α, β-unaturated aldehyde, is by far the most reactive amongst the aldehydes present in smoke. Levels of acrolein in ambient air are estimated to 0.04 to 0.08 ppm, although it is found at up to 90 ppm in cigarette smoke. Subchronic-intubation animal studies have reported physiological effects at 0.4 ppm. The relative contribution of acrolein to complex mixture toxicity of tobacco smoke is not yet known. Exposure to benz(a)pyrene (BaP) increases the expression of human type II (A549) lung cells to acrolein has shown that it inhibits the redox sensitive transcription factors NF-κB and AP-1. We have extended this study to examine the ability of acrolein to modulate the effect of benz(a)pyrene (BaP) on p53 protein. The hypothesis being investigated is that a chiral imbalance induced by acrolein can alter the activity of p53 protein. Exposure of A549 cells to 1 μM BaP for 48 h strongly activated the expression of p53 seen by Western blotting, and its DNA binding as shown by electrophoretic mobility shift assay. Treatment of A549 cells with a non lethal dose of acrolein (50 μmol/cm2) for 0.5 h depleted 80% of total cellular glutathione but did not alter p53 protein levels. When BaP-treated cells (48 h) were exposed to acrolein for 0.5 h there was no effect on BaP-induced p53 protein levels, but a 50% inhibition in the DNA binding of p53 activated by BaP. Using a p53 specific reporter, fosfomycin was administered an 85% decrease in the p53 activity induced by BaP 1 μM (for 24 h post transfection). The downstream consequence of inhibiting p53 activity with acrolein is being investigated. Results from this study suggest that acrolein can alter p53 DNA binding and function which may play an important role in lung cancer attributed to cigarette smoke carcinogens such as BaP. [This work was supported by the Maryland Cigarette Restitution Fund, NIHES Center Grant ES08389 and ROI ES07971)]

1505 FOMESAFEN: A MURINE HEPATOCARCINOGEN THAT ACTS VIA PPARα TO SUPPRESS APOPTOSIS AND INDUCE CELL AND PEROXISOME PROLIFERATION.


Fomesafen is a herbicide that increased liver tumor incidence in male and female CD-1 mice when administered in the diet at 100 and 1000 ppm. Previous studies indicated that fomesafen belongs to the peroxisome proliferator (PP) class of non-genotoxic hepatocarcinogens. PP-1, the peroxisome proliferator activated receptor α (PPARα) causes peroxisome proliferation, suppression of apoptosis and cell proliferation, ultimately leading to tumors. In contrast, human liver is refractory to the adverse effects of PPs. To confirm that fomesafen belongs to the PP class, a non-genotoxic hepatocarcinogen, fomesafen was administered to CD-1 mice for 28 days. Fomesafen at 100 or 1000 ppm increased mean relative liver weight in male and female mice (1.4- and 1.8-fold and 1.1- and 1.6-fold, respectively, relative to control), and caused a marked induction of hepatic peroxisomal volume in both male and female mice (8.2- and 12.0-fold and 9.4- and 12.5-fold, respectively, relative to control). To assess the ability to regulate cell proliferation, and apoptosis, and to assess the role played by PPARα, hepatocytes isolated from control and PPARα null mice were used for in vitro investigations. In wild type mouse hepatocytes, fomesafen (250μM) caused peroxisome proliferation, increased the rate of cell proliferation (from 7.6±0.7% to 12.0±1.9% of cells in S-phase) and decreased the rate of apoptosis (from 1.4±0.2% to 0.7±0.1%). In PPARα null hepatocytes were refractory to the effects of fomesafen on peroxisome proliferation, cell proliferation and apoptosis. Since human liver is resistant to the effects of PPs, the response of human hepatocytes to fomesafen was investigated in vitro. Human hepatocytes were refractory to the effects of fomesafen on peroxisome proliferation, cell proliferation and apoptosis. These data demonstrate that fomesafen belongs to the PP class of rodent specific hepatocarcinogens that induce tumors via a mode of action that is not relevant for man.

1506 INHIBITION OF APOPTOSIS IN RAT HEPATOCYTES BY "NON-DIOXIN-LIKE" POLYCHLORINATED BIPHENYLS.

H. J. Schmitz, B. Wagner, S. Bohmberger and D. Schrenk, Food Chemistry and Environmental Toxicology, University of Kaiserslautern, Kaiserslautern, Germany.

Polychlorinated biphenyl (PCB) lead to a variety of biological effects in experimental models. Among other adverse effects in humans, an increased incidence of liver cancer was observed under high exposure to PCBs. In contrast to 'dioxin-like' PCBs, 'non-dioxin-like' PCBs are inactive or almost inactive as aryl hydrocarbon receptor (AhR) agonists. Although they do not share structural properties with polychlorinated biphenyl (PCB) certain 'non-dioxin-like' PCBs show induction of PB-inducible drug-metabolizing enzymes. Like TCD and PB, several 'non-dioxin-like' PCBs possess promotor activity in rat liver. Since the inhibition of apoptosis in pre-neoplastic foci has been demonstrated for the liver tumor promoters TCD and PB, it may also play a central role in the mechanism of tumor promotion by PCBs. Nevertheless, no data concerning an anti-apoptotic effect of tumor-promoting PCBs in pre-neoplastic rat liver are available. This study investigates the possible role of 'non-dioxin-like' PCBs 'type II' induction of CYP isoforms and the effect on UV-initiated apoptosis in cultured rat hepatocytes after treatment with 'non-dioxin-like' PCBs. For this purpose, hepatocytes from rat liver were cultured using the collagen sandwich technique and apoptosis was initiated by irradiation with UV light. Addition of the PCBs JUPAC numbers 28, 101 and 187 and PCB 30 30 min after irradiation, suppressed the UV-induced increase in apoptosis in a concentration-dependent manner, following the rank order of PCB 28 > PCB 101 >PB > PCB 187 for apoptosis-suppressing potency. PB and all PCBs tested also led to a concentration-dependent induction of CYP2B1/2B2 activity following the rank order PCB 187 > PCB 28 > PCB 101 > PB for inducing potencies. For the first time, our results demonstrate the suppression of UV-initiated apoptosis in rat hepatocytes by 'non-dioxin-like' PCBs.
1507
ROLE OF CHOLINE DEPLETION IN DIETHANOLAMINE INDUCED DNA SYNTHESIS IN MOUSE HEPATOCYTES.
J. M. Karamendulis, D. J. Smith and J. E. Klaunig. Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

Diethanolamine increased the incidence and multiplicity of liver tumors in the mouse but not in the rat following chronic dermal exposure. While the mechanism(s) for tumor induction is not readily evident, diethanolamine inhibits cellular choline uptake. Choline deficiency is known to produce tumors in rodents, therefore, one might postulate that diethanolamine, through depletion of choline, may result in the development of tumors in the mouse. The present studies examined the effect of diethanolamine and choline depletion on DNA synthesis in primary cultured mouse hepatocytes. Mouse hepatocytes were cultured with 0-500 mg/ml diethanolamine for 24 hours and DNA synthesis measured immunohistochemically, based on BrdU incorporation into DNA. In mouse hepatocytes DNA synthesis was increased following treatment with 10 mg/ml diethanolamine and higher and ranged from 1.8- to 3.2-fold over control. Incubation of mouse hepatocytes in medium containing reduced choline concentrations (1/10 to 1/100 of normal medium: 0.898 mg/L to 0.0889 to 8.98 mg/L) for 24 hours resulted in an increase in DNA synthesis (1.4- to 2.4-fold over control); whereas incubation of cells in medium containing 10-fold higher choline concentration produced a 50% reduction in DNA synthesis from control. These results showing that reduction of cellular choline results in an increase in DNA synthesis suggest that the DNA synthesis seen following diethanolamine treatment may be a result of cellular choline depletion and may contribute to diethanolamine carcinogenicity.

1508
ENDOTHELIAL-CELL KINETICS IN RAT MODEL OF RIDDLELLINE-INDUCED HEMANGIOsarcoma.

Riddlelline is a naturally occurring pyrrolizidine alkaloid isolated by the FDA for carcinogenicity testing because of its economic impact on the livestock industry and the potential for human exposure. In NTP 2-year studies with riddlelline administered by gavage, high incidences of hemangio and hemangiosarcoma were induced in the liver of rats and mice. In an effort to understand the pathogenesis of hemangiosarcoma, the capacity of riddlelline to damage endothelial cells and induce endothelial-cell proliferation was tested. 42 male Fisher rats received riddlelline (0, 1, and 2.5 mg/kg/day) for up to 6 weeks. 7 animals/group were sacrificed after 8 consecutive daily doses, and the remainder were sacrificed after 30 doses. By light microscopy, non-parenchymal cells with perivascular and karyomegalic changes were noted in the high-dose livers of the terminal sacrifice. These cells were immunopositive for Factor VIII and were considered endothelial. A slight trend of increase in apoptosis (TUNEL method) was noted in the liver of the high-dose animals of the interim sacrifice. A significant increase in non-parenchymal-cell proliferation (BrdU method) was noted in the liver of animals of the interim and terminal sacrifices. A significant increase in parenchymal-cell proliferation was noted in the liver of the interim-sacrificed, while an opposite effect was noted in the terminal sacrificed. Electron microscopic evaluation indicated treatment-related damage as well as hyperplasia of non-parenchymal cells. The data suggest that the endothelial cells are specifically damaged by riddlelline. This may lead to endothelial-cell proliferation, perhaps of the regenerative type. Possible growth factors involved in the proliferation of the endothelial cells are currently being investigated.

1509
15-DEOXY-2',14'-PROSTAGLANDIN J2, A LIGAND OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-Y, INDUCED APOPTOSIS THROUGH G2/M PHASE ARREST IN NEOBRIOBLASTOMA CELLS.
K. S. Park, F. J. Kim, J. H. Oh, C. W. Seong, H. K. Jung, K. H. Yang, S. Y. Chung, Y. Y. Sheen and J. T. Hong. Department of General Toxicology, National Toxicol Res., Seoul, South Korea, College of Pharmacy, Ewha Womans University, Seoul, South Korea and College of Pharmacy, Changwon National University, Changwon, South Korea.

15-Deoxy-Δ15-15-Di3,14-Prostaglandin J2 (15-deoxy-PGJ2), a peroxisome proliferator-activated receptor (PPAR) ligand, has been shown to stimulate differentiation and induced apoptosis in several cancer cells including breast, prostate and lung cancer cells. In this study, we examined whether 15-deoxy-PGJ2 could induce cell growth through induction of apoptosis in neuroblastoma cells (SK-N-MC and SK-N-SF). We also investigated the expression of (p53) and apoptosis-related genes and activation of transcription factors. 15-Deoxy-PGJ2 inhibited neuroblastoma cell growth and induced apoptosis in a dose (2-16 μM) and time-dependent manner. Consistent with the induction of apoptosis, 15-deoxy-PGJ2 reduced the expression of anti-apoptotic Bcl-2 but increased the expression of pro-apoptotic Bax in phase 3 and phase 9. Flow cytometric analysis revealed these cells were arrested in G2/M phase after 15-deoxy-PGJ2 treatment. Furthermore, 15-deoxy-PGJ2 significantly increased the expression of cyclin B1, but decreased the expression of cdK1, cyclin D1, cdK2 and cdK2. It was also found that FAPAR-γ2 was expressed by 15-deoxy-PGJ2 in these cells. Taken together, these results suggest that 15-deoxy-PGJ2 may be a candidate for a preventive or a therapeutic agent for neuroblastoma.

1510
INHIBITION OF UV-B-MEDIATED ACTIVATION OF NUCLEAR FACTOR KB BY GREEN TEA CONSTITUENT-(3)-EPICALLOTHECHIN-3-GALLATE IN CULTURED HUMAN EPIDERMAL KERATINOCYTES.
E. Aafa, V. M. Adhami, N. Ahmad and H. Muhitar. Dermatology Case Western Reserve University, Cleveland, OH.

UVB (U) radiation to mammalian skin results in oxidative stress via an increased generation of reactive oxygen species (ROS). ROS is also known to cause a variety of adverse effects including cancer. Nuclear factor kappa B (NF-kB), a redox sensitive transcription factor, plays an important role in regulating the expression of various genes that participate in many physiological processes such as inflammation, apoptosis, and cellular proliferation. Green tea polyphenol (-epicallothechin-3-gallate (EGCG) has been shown to have photoprotective properties. Because, NF-kB has been implicated in UV response, we investigated its involvement in photochoprotective effects of EGCG. In dose- and time-dependent studies, we evaluated the effect of EGCG on the phosphorylation and degradation of IkB alpha and activation of IKK alpha and NF-kB following UVB irradiation of normal human epidermal keratinocytes (NHEK). Immunoblot analysis demonstrated that the treatment of NHEK with EGCG (10-40 micro M) for 24 hrs before UVB (40 nJ/cm2) exposure dose-dependently inhibited UVB-mediated degradation and phosphorylation of IkB alpha and activation of IKK alpha protein expression. Employing immunoblot analysis and enzyme linked immunoassortent assay, we found that EGCG inhibits the UVB-mediated activation of NF-kB/p65 in the nucleus. In time-dependent study we observed that EGCG (10 micro M) inhibits degradation and phosphorylation of IkB alpha and activation of IKK alpha and NF-kB/p65 at 1, 2, 3, 6, 12 hrs post UVB-exposure. Next we examined the effect of UVB on upstream targets of NF-kB. Following UVB-exposure, the expression of RANK (receptor activator of NF-kB) and NIK (NF-kB-inducing kinase) proteins remained unchanged suggesting that RANK and NIK are not involved in UVB-mediated activation of NF-kB. These results provide molecular basis for the photoprotective effects of EGCG and suggest that green tea may be a useful agent against UVB-induced effect in human skin.

1511
MODULATION OF TRANSFORMED AND NORMAL SYRIAN HAMSTER EMBRYO (SHE) CELL GROWTH BY COMPONENTS OF GREEN AND BLACK TEA.
Y. Xu, Z. Jiao and J. F. Klaunig. Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

The field of chemoprevention has seen increased interest in the last decade. While a number of chemicals have been shown to delay or prevent the cancer process, less is known about the mechanisms by which chemopreventive agents function in this regard. Chemicals can inhibit a tumorigenic growth by function directly on the precancerous, initiated cells or indirectly on normal cells surrounding them. Using benzo(a)pyrene (BP) induced transformed SHE: cells and untreated, normal SHE cells, we studied the effects of a component of green tea, EGCG, and of black tea, tea pigments, on the growth and death of premalignant and normal SHE cells. Following treatment both EGCG and tea pigment increased the clonal growth rate of normal SHE cells in a dose dependent manner. In contrast, a decrease of clonal growth of the BP transformed cells was observed. Further investigation, showed that the decreased clonal growth of the BP induced premalignant SHE cells was through an increase in cell death (apoptosis). These data suggest a selective effect on cell growth and death of the tea components on the premalignant and normal SHE cells. This effect was dose responsive in both cell types. No difference was seen at low doses of the components and at high doses, the tea components decreased the growth of both normal and premalignant cells. In addition, an increase in DNA damage (using Comet analysis) was seen at high doses of the tea components in both cell types suggesting a risk to promote the growth of transformed cells. These dose-related modifications growth regulation of the tea components in gene express-
**1512**

**INDUCTION OF QUINONE REDUCTASE ACTIVITY IN HEPA 1C1C7 CELLS AND IN FISHER 344 RATS BY BROCCOLI DEPENDS ON SULFOPHANE BUT NOT ON GLUCORAPHANIN CONTENT.**

A. S. Keck and E. H. Jeffrey. *Division of Nutrition, Champaign-Urbana, IL.*

Sulforaphanes (GS) and their bioactive hydrolys products such as sulfophane (SF) upregulate detoxification enzymes and are suggested to be responsible for the chemopreventive effects of cruciferous vegetables. Broccoli (brassica oleracea L. , Italica) is the primary source of GS in the American diet. We previously anarlyzed the sulforaphane levels in 50 broccoli varieties and found that the total GS content and individual GS species varied greatly depending on variety. In this study nine broccoli varieties were tested for their ability to increase quinone reductase (QR) activity, a biomarker for anti-carcinogenesis, in mouse hepatoma (HePa 1C1C7) cells. The objective was to study whether QR activity correlates with total amount of GS, the specific GS glucoraphanin (GP, precursor to SF) or any of the hydrolys products such as SF. 150,000 cells/well were plated in 12 well plates 24 h prior to exposure to aqueous extracts of 100, 500 or 1000 mug freeze-dried broccoli/ml medium, for 24 h. The increase of QR activity was dose-dependent and significant differences in QR incidence among the broccoli varieties were detected: Pest #7 > Mal 191 > Pest #15 > Packman > Majestic > Sungold > V1-158 > EV-6-1. QR induction did not correlate with total GS or GP, but did correlate significantly (p<0.01) with SF content of the extracts. Two broccoli varieties, Packman and Majestic, were fed to male Fisher 344 rats for 5 days (20% of diet). As in cell culture, hepatic QR induction reflected relative SF levels, but not total GS or GP levels. Results indicate that SF is primarily responsible for increases of QR activity both in vitro and in vivo and that different broccoli varieties may provide differing chemopreventive potency. Supported by USDA grant # 99-35503-7010

**1513**

**IN VITRO TWO-STAGE MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO (SHE) CELL.**

H. Zhang, H. D. Bornman and B. C. Meier. *Genetic and Molecular Toxicology, Corvus Laboratories Inc., Vienna, VA.*

Cell transformation systems have been used for many years to study the mechanisms of carcinogenesis in *vivo* and to detect the carcinogenic potential of chemicals. Cell transformation to a malignant state is a multistage process, and in SHE cells the first stage is characterized by a morphological transformation (MT). MT may itself be susceptible to a generalized two-stage process of initiation and promotion, as that occurs in vivo. Although two-stage transformation models have been demonstrated in several cell transformation systems and exploratory work has been performed with SHE cells, a well-defined method for detecting initiating/promoting activity in SHE cell cultures has not been developed. The initial results of a program to establish an in vitro two-stage SHE cell morphological transformation assay system are reported. BaP and TPA were used as an initiator and promoter, respectively. A preliminary study was conducted to determine subtransforming doses of BaP and TPA. No significant increases in the frequency of MT were observed in SHE cell cultures treated with BaP for 24 hours at <334 ng/ml. Treatment with TPA for 7 days at 1 ng/ml also did not induce significant increases in the MT frequency. When SHE cell cultures were treated with 334 ng/ml BaP for 24 hours, followed by TPA at 1 ng/ml for 6 days, a MT frequency of 2.18% was observed, compared to 0.63% by BaP treatment alone and 0.56% by TPA alone. Thus, a synergistic effect appeared to occur between the two subtransforming treatments. Similarly, a synergistic effect on MT was also observed in SHE cell cultures treated with BaP at a transforming dose of 5 ng/ml for 6 days. The MT frequency induced by the sequential treatment was 2.98%, with a frequency of 1.79% for BaP alone and 0.29% for TPA alone. These initial data suggest that a two-stage morphological transformation model can be established using cultured SHE cells and studies designed to fully characterize this model system are in progress.

**1514**

**DEVELOPMENT OF TUMOR PROMOTER TESTING MODEL WITH TRANSFECTED CELL LINES HARBORING C-HA-RAS/12G.**

T. Wu and M. Yin. *Protein Engineering, Beijing Institute of Biochemistry, Beijing 100071, China.*

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**1515**

**THE ABSENCE OF FASL ENHANCES THE DEVELOPMENT OF TUMORS AND REDUCES LIFE EXPECTANCY OF P53+/− MICE.**

M. Embree-Ku and K. Bockelheide. *Pathology and Laboratory Medicine, Brown University, Providence, RI.*

In the multi-step process of tumor development, several genetic and epigenetic events occur to transform cells from normal to malignant. Although p53 is one of the most commonly mutated genes in a wide variety of tumors, how other genes interact with p53 to transform cells is only just beginning to be understood. To study the effects of the interaction of the Fas system with p53 in tumor progression and development, mice with a targeted disruption of the p53 tumor suppressor gene and deficient in Fas ligand were evaluated. Organ weights, life expectancy, and tumor and tissue histology were assessed. Although the absence of Fasl had no effect on life expectancy of homozygous p53-deficient mice, the absence of a functional Fasl had a profound effect on the life expectancy of p53 heterozygous mice, reducing the median time to death from 12.1 months to 9.6 months. Additionally, the tumor spectrum in p53 heterozygous mice shifted from predominantly sarcomas (63%) when Fasl was present to a large number of lymphomas (76%) in Fasl−/− p53−− mice. The reduction in life span and increased incidence of lymphomas in Fasl−/− p53−− mice suggests that these mice could be used in carcinogenesis testing, particularly in understanding the mechanisms of compounds that induce lymphomas. Future studies will examine the possibility of using Fasl−/− p53−− mice as an alternative model in carcinogenesis assessment.

**1516**

**EVALUATION OF THE Tg.AC TRANSGENIC MOUSE FOR PREDICTING THE PHOTOCARCINOGENIC POTENTIAL OF PHARMACEUTICALS. DOSE-RESPONSE OF Tg.AC MICE TO SOLAR-SIMULATED LIGHT.**

R. Honchiell, J. Zhang, C. S. Trempeus, S. A. Miller, G. N. Rao and F. D. Staszak.* "CDER, FDA, Laurel, MD and "NIEHS, NIH, Research Triangle Park, NC.*

Tg.AC mice [FVB/N-Tg(ha-ras)] have been shown to develop large numbers of papillomas within 26 weeks in response to dermal application of certain carcinogens. This propensity to develop skin tumors may make Tg.AC mice an ideal alternative to the current short-term model for photocarcinogenic testing. Since UV light alone is a carcinogen, our first objective was to determine the threshold levels of solar simulated light (SSL) that are tumorigenic to Tg.AC mice. The acute minimal erythema dose (MED) was determined in FVB/N mice (the parental strain). Groups of male and female Tg.AC mice were then exposed to a fraction of the MED of SSL (0.0, 0.1, 0.2, 0.3, 0.4, or 0.6 MED). A similar SSL dose-response study was performed on FVB/N mice to confirm an enhanced tumorigenic response in Tg.AC mice. Mice were exposed 5 days per week for 26 weeks, then observed for an additional 12 weeks with no SSL exposure. No significant papilloma incidence was observed with any group after 26 weeks of treatment with SSL or after 12 additional weeks of observation. Most Tg.AC mice that received the higher SSL doses developed large nonpapilloma skin tumors (4/6 male and 5/6 female in 0.6 MED group vs 0/4 male and 0/6 female in no SSL group), but only during the 12 week post SSL dosing period of the study. In FVB/N mice, only the female 0.6 MED group (2/7) developed skin tumors (both nonpapillomas) during the final week (week 38). Unlike typical carcinogen-induced tumors in Tg.AC mice, in situ hyperplasia, hyperplasia, and carcinoma in situ occurred. Unexpectedly, the thenic was not expressed in these UV-induced tumors and immunochemistry analysis revealed that all tumors were positive for p53 expression. The absence of skin tumors in all SSL treatment groups at 26 weeks indicates...
that Tg-AC mice are not highly sensitive to chronic suberythemal SSL exposure and that the “background” incidence will be low when evaluating the Tg-AC mouse response to photocarcinogens in future studies.

1517

COMPARISON OF THE SKH1 AND K6/ODC MOUSE STRAINS FOR SENSITIVITY TO PHOTOCARCINOGENESIS

Y. Chen and T. G. O'Brien. ODC Mouse Group, Drexel Hill, PA

As a result of overexpression of the enzyme ornithine decarboxylase (ODC) in skin, the K6/ODC transgenic mouse is exquisitely sensitive to tumor induction by a wide variety of genotoxic chemicals. In order to determine this model’s sensitivity to UV-induced carcinogenesis, K6/ODC mice and the widely used photocarcinogenesis model, SKH1, were subjected to a side-by-side comparison experiment. Groups of mice were exposed to 4 dose levels of primarily UVB radiation from the same bank of U-Panel lamps on the same days of the week. Mice were irradiated three times a week for 24 weeks. The highest dose (100 mJ/cm^2 3 times per week) corresponded to a weekly dose of 5 minimal erythema doses (MEDs). At the highest dose, K6/ODC and SKH1 mice developed an equivalent tumor incidence (75%) and multiplicity (1.5 tumors/mouse). At progressively lower doses, however, K6/ODC mice developed more tumors than SKH1. At 60 mJ/cm^2, thrice weekly, the tumor incidence was 45% vs. 15% and multiplicity was 0.45 vs. 0.15 for K6/ODC and SKH1 mice, respectively. At the two lowest doses (30 and 15 mJ/cm^2, thrice weekly), SKH1 mice developed no tumors while K6/ODC mice had 5 tumors in these dose groups. There were no tumors in unirradiated control groups of either strain. The majority of the tumors were squamous papillomas in both strains, but some squamous cell carcinomas were also present. These results demonstrate the utility of the K6/ODC model for photocarcinogenesis studies, especially at low doses and relatively short exposure periods. Because of its high sensitivity to both genotoxic chemicals and UV irradiation, the K6/ODC mouse may be uniquely suited for certain applications, such as studies of the interaction of environmental chemicals and UV. (Supported by SBIR grant K43 CA 76081 from NCI, NIH, DHHS)

1518

HISTOPATHOLOGIC EVALUATION OF CLARA CELL-DERIVED LUNG TUMOR PROGRESSION IN A TRANSGENIC MOUSE MODEL


The purpose of the present work was to characterize the histomorphological progression and phenotype of Clara cell-derived lung tumors in a transgenic mouse. In this model, the Clara cell secretary protein (CC10) promoter was used to direct expression of the SV40 large T antigen (SV40TAg) to Clara cells. These mice developed lung tumors within 3 months. Early lesions observed at 1 month included terminal bronchioles lined by hyperplastic, intensely basophilic, cuboidal epithelial cells, which were disorganized and formed small papillary projections. Bronchiolar epithelium was also extending into adjacent alveoli. In larger airways there were multilobal segments of basophilic disorganized cuboidal epithelium interspersed between segments of normal ciliated epithelium. At this time, CC10 expression was markedly reduced as determined by Western blotting and immunohistochemistry. Lung lesions advanced with time, and at 2 months there was a more prominent extension of proliferating epithelium into alveoli. Solid masses of cuboidal cells were forming in terminal bronchioi and papillary lesions were present in medium to larger bronchioles. By 3 months, solid tumor masses were starting to compress adjacent alveoli and there were focal areas of disorganized small alveolar cells. At 4 months, tumor size increased with obvious compression of adjacent alveoli and there was evidence of pleural fibrosis and pleural lymphatic invasion. This transgenic mouse model, characterized by extensive bronchiolar hyperplasia which progresses rapidly to tumor formation, provides new insight into changes associated with Clara cell transformation and differentiation. The model also demonstrates the early loss of CC10 expression in Clara cell-derived tumors.

1519

DOXYCYCLINE (DOX)-REGULATED, LUNG-SPECIFIC EXPRESSION OF MUTANT HUMAN KI-nas IN BIOTRANSGENIC MICE: ALTERATIONS IN LUNG MORPHOLOGY

H. M. Smith, J. W. Tchelara, C. M. Mikesko, S. T. Dance, J. Eversn, J. A. Whitson and M. S. Miller. "Wake Forest University School of Medicine, Winston-Salem, NC, "Children's Hospital Research Foundation, Cincinnati, OH and "CIIT, Research Triangle Park, NC.

Mutation in KI-nas is a critical and early event in the development of lung adenocarcinomas in both humans and animal tumor models. Development of transgenic mice that possess constitutively active KI-nas has been attempted but was unsuccessful because expression of the transgene resulted in embryonic lethality. We have thus developed a biotransgenic mouse model that allows for the regulation of KI-nas expression in a lung-specific and DOX-inducible manner. A CYS mutant allele of the human KI-nas gene was linked to a tetracycline (tet)-inducible promoter and then used to establish a line of transgenic mice with this construct. These mice, which do not express the KI-nas gene, were then crossed with a second transgenic line containing the reverse tet activator (rTnA) expressed constitutively from either the SPC or CC10 lung-specific promoters. Induction of the KI-nas transgene was detected by RT-PCR in biotransgenic mice following 1 week of DOX exposure. DOX-treated biotransgenic mice exhibited hyperplastic lung foci after only 12 days of DOX-treatment. By 5 weeks of treatment, extensive epithelial hyperplasia of the alveolar region of the lung tissue could be seen. Lung morphology in untreated biotransgenic mice or either of the DOX-treated single transgenic or control mice was normal. The hyperplasia noted in the lungs is consistent with peneophleptic lesions observed in chemically-induced mouse lung tumors that precede the onset of frank neoplasia. These results demonstrate that the biotransgenic model will provide an important new research tool that will allow us to determine the role of KI-nas mutations in lung tumorigenesis. This will have important implications for future studies on the etiology, genetics, prevention, and development of novel therapies for lung cancer.

1520

REPLACEMENT OF PARTIAL HEPATECTOMY WITH FASTING/REFEEDING OR THE USE OF NEONATAL ANIMALS IN THE RESISTANT HEPATOCYTE MODEL

P. Espandari, H. P. Clauert and L. W. Robertson. Toxicology, University of Kentucky, Lexington, KY

In rat liver several models have been developed to study multistage carcinogenesis, including the Solte-Farber resistant hepatocyte model. In this model initiation consists of either a necrogenic dose of a hepatocarcinogen or a non-necrogenic dose in conjunction with partial hepatectomy. As an alternative to partial hepatectomy, we investigated whether a different procedure, fasting/refeeding or the use of one-day-old neonates, would provide an alternative protocol. The experimental design consisted of four groups: 1) control; 2) fasted for 4 days, then refeed; 3) partial hepatectomy; 4) neonates. Groups 1-3 were injected p.o. with diethylstilbestrol (DEN) (100 mg/kg) 24 hrs after refeeding or partial hepatectomy. Group 4 was treated with the same dose of DEN at one day of age. All initiation animals were treated with selection agents; 3 daily doses of 2-acetylaminofluorene (2-AF), followed by a single dose of carbon tetrachloride (2ml/kg) followed by 3 additional daily treatments of 2-AA. Rats were killed two weeks after the last treatment. Most of the rats in the partial hepatectomy and fasting/refeeding groups developed visible nodules. All three experimental groups developed significantly more gamma-glutamyl transpeptidase (GGT)-positive foci per cu cm of liver tissue than in the control group. In the fasting/refeeding group, the size and the volume % of GGT-positive foci in liver tissues were higher compared to rats subjected to partial hepatectomy. Furthermore, the number of GGT-positive foci in neonates was higher compared to the partial hepatectomy group. We conclude that both fasting/refeeding and the use of neonatal rats can replace partial hepatectomy as a method of initiation in the Solte-Farber method since both of these methods appear to be less stressful to the animals and equally sensitive. (Supported by ES 07380)

1521

ALTERED LEVELS OF PITUITARY AND SEX HORMONES IN MEXICAN AGRICULTURAL WORKERS EXPOSED TO ORGANOPHOSPHATE PESTICIDES


Many pesticides are considered endocrine disruptors due to their capacity to block or activate steroid hormone receptors and/or to affect reproductive hormone levels in experimental models. However, information regarding organophosphate pesticide (OP) exposure and their effects on humans is limited. The objective of this study was to evaluate the relationships between pituitary and sex hormone levels and OP exposure. A longitudinal study was conducted in an agricultural community located in north Mexico where OP were the most widely sold and used pesticides. Hormone levels were measured according to WHO. OP exposure was evaluated by measuring urinary levels of six dialkyl phosphates and phosphorothiates and by an exposure index constructed on the basis of frequency and intensity of OP exposure. PSH serum levels were negatively associated with OP metabolites DMTP and DMDTP. FSH levels were also significantly decreased in the most exposed group. LH serum levels were also negatively associated with DMDTP and DMDTP. Marginal decreases in prolactin levels were also observed. Regarding sexual hormones, significant increases in estradiol serum levels were observed in the periods of high exposure and they were positively associated with DMP and DEPTP urinary concentrations. Testosterone levels increased during the period of highest exposure and were positively associated with DMTTP concentrations. Our results strongly suggest that high OP exposure alters the homeostatic relationships between the pi
1522
NEUROBEHAVIORAL EVALUATION OF RESIDUAL EFFECTS OF LOW-LEVEL BYSTANDER ORGANOPHOSPHATE PESTICIDE EXPOSURE.
R. Singer, Independent practice, Santa Fe, NM.
Organophosphate pesticides (OPs) may cause neurotoxicity at low-levels of exposure. Subject: A female, 42 years old, high school graduate, farmer, working in a hay field, wearing shorts and a swim suit top, without respiratory protection was exposed to Thimet and possibly Lorsban applied to a downwind neighboring field in 6/98, with re-exposure by working in the adjacent field 6-8 times over the next 3 days. The evening of the exposure, she experienced headache, nausea, vomiting, leg pain, diarrhea, chest tightness, dizziness, blurred vision, and weakness in legs and arms - symptoms of OP poisoning - with continuing vertigo, nausea, pain etc. Later and persistent symptoms included memory loss, sleep disorder, fatigue, irritability, and impaired executive function. ACHE testing was inconclusive. Assessment: Included comprehensive neurobehavioral toxicity evaluations, 2 years post-exposure, with an extensive interview and testing, and record review (educational, medical, letters of reference). Findings: Prior IQ was at the 89th percentile. The Neurotoxicity Screening Survey showed results consistent with those of patients diagnosed with neurotoxicity. Current full-scale IQ had declined to the 47th percentile, with Processing Speed (a factor very sensitive to global neurotoxicity) at the 89th percentile. Additional deficits were seen in detecting visual figure-ground relationships (36th percentile); Selective Reminding Test (measures learning) <10th percentile; Stroop Color and Word Test (measures mental flexibility) <10th percentile; Visual Search and Attention Test <20th percentile; logical memory <10th percentile; with moderate anxiety and moderate-severe depression. Distortion was below the level of detection. Malingering was ruled out by 5 separate tests. Personality testing using the Minnesota Multiphasic Inventory found no personality disorders that could contribute to the findings. Record review found no competing explanations of her illness. Conclusion: Relatively low-level organophosphate exposure can cause neurotoxicity, revealed by neurobehavioral evaluation, lasting many years after exposure.

1523
AN APPROACH FOR SCREENING CHOLINESTERASE INHIBITORS IN DRINKING WATER.
Under the 1996 amended Safe Drinking Water Act (SDWA), a Contaminant Candidates List (CCL) has been completed in 1998 (FR 63:40). Several Cholinesterase (ChE) inhibitors were identified in this list, some are organophosphates and others are carbamates. Prior to 1996, ChE inhibitors in drinking water were regulated individually and monitored by using conventional analytical methods to detect the presence and concentration of individual compounds. Because of specific advances in analytical chemistry and the need for cumulative risk assessment of chemicals that have a common mechanism of toxicity, we developed an approach for initial screening for the presence of ChE inhibitors in drinking water. This approach is based on the use of a Microwell Plate Assay kit. In this method, Acetyl Cholinesterase (ACHE) was stabilized in a gelatin film to determine low levels of pesticide contaminants in drinking water that inhibit ChE activity. The remarkable properties of the dry immobilized ChE preparation include its stability to prolonged storage at room temperature as well as its stability to short term elevated temperatures (60 degrees C). The enzyme could be maintained in dry gel form for 365 days at room temperature without substantial loss of activity. Several procedures were evaluated to oxidize less potent organophosphate (P-S) compounds to their more inhibitory oxon forms. Inhibition profiles were run for six commonly used carbamate insecticides (and some of their metabolites) and eight organophosphate insecticides using this assay. IC50 and IC90 values were determined for purified water and several drinking water matrices. Results using this assay were also compared with commercially available test kits. This method may be successfully used for screening drinking water ChE inhibitors individually or in mixture. The opinions expressed are those of the authors and do not necessarily reflect USEPA policy.

1524
ACETYLCHOLINESTERASE INHIBITION: PREDICTION USING THREE-DIMENSIONAL QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS.
Neurotoxicology, National Center for Toxicological Research/USFDA, Jefferson, AR and Molecular Simulations Inc., San Diego, CA.
Organophosphorus pesticides are known to produce their biological effects through the inhibition of a number of esterases, including acetylcholinesterase (ACHE), the enzyme responsible for the degradation of the neurotransmitter acetylcholine. The combined use of conformational analysis and three dimensional (3D), quantitative structure-activity relationship (QSAR) methods were used to rationalize the inhibitory potencies of a series of organophosphorus pesticides against AChE. The CNT-ALYSi program was used to identify the structural features in the group of eight inhibitors whose IC50 values ranged from 0.34 nM to 1.2 μM. The 3-D pharmacophore models were characterized by at least one hydrogen bond acceptor site and 2-3 hydrophobic sites and demonstrated good correlation (r2 = 0.994) between the predicted and experimental IC50 values. This approach can be used to screen databases of organophosphorus and other chemical categories for their neurotoxic potential via the inhibition of AChE.

1525
ASSESSING THE IMPACT OF HUMAN PON1 POLYMORPHISMS: SENSITIVITY AND MONTE CARLO ANALYSES USING A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC (PBPK/PD) MODEL FOR CHLORPYRIFOS.
C. Timchalk, A. Kousha and T. S. Post.
Barrel, Pacific Northwest Division, Richland, WA.
A PBPK/PD model was developed for chlorpyrifos (CPF) and the active metabolite CPF-oxon. Susceptibility to organophosphate (OP) insecticides is associated with variation in pharmacokinetic/pharmacodynamic response. A genetic polymorphism in the PON1 (acylhydrolase) deactivation of OP results in the expression of a range of PON1 enzyme activities within humans. The objective was to identify sensitive parameters that influence the model output, and to investigate the impact of human PON1 status on CPF-oxon brain concentration over a range (3 μg/g - 5 mg/kg) of CPF doses. The model was used for parameter sensitivity analysis, and Monte Carlo simulations for PON1 activity utilizing the human PON1 polymorphic distributions. Based on the sensitivity coefficients (SC), the model was sensitive to the following parameters: CPF-oxon plasma protein-binding, liver partitioning, liver PON1 metabolism, plasma butyrylcholinesterase (BuChE) and blood flow to the liver and brain. In addition, the SC for plasma protein binding, PON1, and BuChE demonstrated a clear dose-dependency. The model simulations suggest a dose-dependent non-linear increase in the brain CPF-oxon: AUC, at doses >0 mg/kg, which is a function of both dose and PON1 activity. In contrast, at low environmentally relevant doses (5-250 μg/kg) the model is relatively insensitive to the variability in PON1 activity. These results suggest that other esterase detoxification pathways may adequately compensate for lower PON1 activity; hence an increased sensitivity to low PON1 is not observable until non-target esterases have been appreciably depleted. This response is consistent with simulations that show an increased sensitivity to PON1 status at doses that significantly deplete plasma BuChE activity in humans (50% inhibited). This study illustrates the utility of PBPK/PD modeling for determining the overall impact of parameter uncertainty and variability on risk assessment predictions for OP insecticides. (Supported by EPA grant R828608)

1526
EFFECTS OF DIETARY CHLORPYRIFOS ON PERIPHERAL TISSUE VERSUS BRAIN AND RBC ACETYLCHOLINESTERASE ACTIVITY IN DOGS.
The Dow Chemical Company, Midland, MI; Dow AgroSciences, Indianapolis, IN and Dow AgroSciences, Wantage, United Kingdom.
Chlorpyrifos (CPF) is a widely-used broad-spectrum insecticide. At the request of the United Kingdom’s Advisory Committee on Pesticides, a 6-week dog study was conducted to examine the effects of dietary exposure to CPF on peripheral tissue (left atrium, nodose ganglia, diaphragm, and quadriiceps muscle) acetylcholinesterase (ACHE) activity as compared to AChE in brain and red blood cells (RBC). Six-month old Beagle dogs (n = 4/sex/group) were given 0, 0.5, 1.0, or 2.0 mg/kg/day CPF in their diets. AChE was assayed using acetylatedcholine in the presence of the butyrylcholinesterase inhibitor iso-OMPA (0.1 mM). AChE activities were compared using Dunn's test (alpha < 0.05). RBC AChE showed a slight (10%) but statistically significant decrease in the mid- and high-dose groups after 1 day of exposure to CPF. RBC AChE was inhibited at all dose levels after one week, reaching a steady-state of inhibition within three weeks of exposure. After 6 weeks of treatment at 2.0 mg/kg/day, a small difference in brain AChE (2% inhibition in males and females combined) may represent a true treatment effect but was not statistically significant and was not considered adverse (-20% inhibition). Peripheral tissue AChE activity was corrected for connective tissue content as AChE activity in diaphragm, quadriiceps, and nodose ganglia was inversely-correlated with the amount of connective tissue in the samples. No statistically significant differences in peripheral tissue AChE were found. However, the left atrium AChE activity in high-dose males was about 25% lower than controls, and may represent a true treatment effect despite a comparable but opposite effect in females, which indicated random variability. We conclude that RBC AChE is more
sensitive than peripheral tissue or brain to inhibition by CFPE. The acute RBC NOAE was 2.0 mg/kg and the repeated-dose NOAE for brain and peripheral tissue was at least 1.0 mg/kg/day.

1527 METHODS TO EVALUATE PERIPHERAL TISSUE ACETYLCOLINESTERASE ACTIVITY IN DOGS TREATED WITH CHLORPYRIFOS.


Total cholinesterase activity (ChE), as measured by reaction with acetylthiocholine (AcCh), is often used as an estimate of acetylcholinesterase activity (ACHE) in animals treated with anticholinesterases. Measurements of ChE may overestimate the potential effects of anticholinesterases on ChE by ignoring the contribution of tissue butyrylcholinesterase (BuChE), for which AcCh is also a substrate. Moreover, the relative content of AChe and BuChE in peripheral tissues is not fully understood in many species, including dogs. Therefore, methods were optimized to measure tissue AChe activity in dogs treated with the anticholinesterase chlorpyrifos (CPF). ChE activity was evaluated in the RBC, brain, and lung, forage, and liver. Sensitivity in RBC AChe activity was at least 1.0 mg/kg/day in their diets for 4 weeks. ChE and BuChE were measured by reaction with AcCh and butyrylthiocholine, respectively. AChe activity was isolated from ChE using iso-OMPA, a specific inhibitor of BuChE. Brain ChE activity was largely inhibited (70% and 80%) in male rats exposed to CPF at 5 mg/kg at postnatal days (PND) 3 and 10. Rats were injected with either corn oil or the specific ChE inhibitor, bis-(4-nitrophenyl) phosphonate (BNPP), followed by injections of CPF or PO. OP dosage was as follows for PND3 and 10, respectively: CPF (0.5 and 15 mg/kg), and PO (0.1 and 1.5 mg/kg). At PND3, brain ChE activity was inhibited by 70% and 81%, in male rats exposed to CPF and BNPP, followed by exposure to CPF or PO, as compared to 44% and 17% for CPF or PO treatment of control rats, respectively. At PND3, activity was similar for males and females. At PND80, ChE inhibition was 84% and 95% in males exposed to BNPP-CPO or BNPP-PO, respectively. However, activity was similar in corn oil control levels for PO exposure, but was inhibited after CPF exposure in males. For PND80 female brain, ChE inhibition was 88% and 90%, in rats exposed to CPF-BNPP and BNPP-PO, respectively, and 57% and 70% inhibited for CPF or PO exposures. The protective effect of CaE can be shown in the difference between ChE activities in the presence and absence of CaE. To evaluate an AEST protection in vivo, and using in vitro inhibition data, it is possible to calculate the effective OP concentration required for the observed inhibition in vivo, and to compare this to the dosage (in micromoles/kg) administered to BNPP-treated animals. For PND3 males, these calculated values [in ratios of nM/micromoles/kg] for CPF and PO were 3.6 for PO and 5.9 for CPF. For PND38 males, which have much higher AEST levels, the values were 3.6 for PO and 5.9 for CPF. These values suggest that AEST are more important for CPF hydrolysis, whereas CaE are more effective in detoxification of both CPF and PO. (Supported by NIH F31 ES05752).

1528 DEVELOPMENT OF AN IN VITRO ASSAY WHICH MAY IDENTIFY THOSE ORGANOPHOSPHORUS PESTICIDES ARE MORE TOXIC TO THE YOUNG.

S. Padilla, H. J. Sung, R Phillips, L. Jackson, and V. Moser. *Neurotoxicology Division, USFPA, Research Triangle Park, NC; Pesticide Safety Division, National Institute of Agricultural Science and Technology, Rural Develop Admin., Ssunsun, South Korea; and Univ. of NC, Chapel Hill, NC.

Some, but not all, organophosphorus pesticides are more acutely toxic to the young as compared to adults. We have developed an in vitro assay which measures the detoxification potential (via carboxylesterase and A-esterase) of tissues. Previous results using this in vitro assay have correlated with the in vivo sensitivity of the young to chlorpyrifos, and also correlated with the equal sensitivity of the young and adult to methamidophos (Padilla et al., *Neurotoxicology*, 2000). We have now extended these observations to other two pesticides that have already been shown in the literature to be more toxic to the young: paraxon and malathion. In our in vitro assay, liver and plasma from 7-day-old rats were much less efficacious at detoxifying the active metabolites of these two pesticides—a pattern that correlates with the in vivo sensitivity of the young to these two pesticides. Using our in vitro assay we also tested the active metabolite of diazinon, diazinon, and again found that young liver or plasma possessed much less detoxification capability than adult tissues. From these results, we predicted that young animals would be more sensitive to diazinon, which, in fact, was the case: when postnatal day (PND) 17 or adult rats were given a dosage of 75 mg/kg diazinon, adult brain cholinesterase (ChE) activity was only inhibited 38%, while brain ChE in the PND17 animals showed much more inhibition (75%). Furthermore, higher doses (200-500 mg/kg) were lethal to PND 17 rats, but adults only showed signs of toxicity. We conclude that our in vitro test is a useful, quick convenient method for predicting in vivo age-related sensitivity to organophosphorus pesticides. This abstract does not reflect EPA policy.

1529 LACK OF SEX DIFFERENCES IN ACETYLCOLINESTERASE INHIBITION IN NEONATAL RAT FOREBRAIN FOLLOWING ACUTE AND REPEATED ORAL EXPOSURE TO CHLORPYRIFOS.

A. M. Betancourt and R. L. Carr, Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

Chlorpyrifos (CPF), one of the most widely used organophosphorus insecticides in the USA, exerts its toxicity through the inhibition of acetylcholinesterase (ACHE). Multiple studies have reported a marked susceptibility of young animals to the toxic effects of CPF compared to adult animals, which has evoked increased concerns about its safety. The literature has reported significant sex differences in the inhibition of ACHE following acute exposure to CPF in neonatal rats. In this study, postnatal day 1 (PND1) male and female rat pups were acutely exposed by oral gavage to 1.5 or 3.0 mg/kg CPF. ACHE activity was determined in the forebrain at various times following exposure to determine the time of peak inhibition of ACHE to identify any possible differences between sexes. Following 1.5 mg/kg CPF, peak inhibition (57-59%) occurred at 12 hours with minor recovery of activity by 24 hours (47-50%). Following 3.0 mg/kg peak inhibition (87%) occurred at 3-4 hours with little recovery by 12 hours but significant recovery by 24 hours (51-56%). Timing of peak inhibition and inhibition levels were very similar for both males and females suggesting that following an acute oral exposure, male and female neonates respond similarly. In addition, male and female rat pups were repeatedly exposed from PND1-PNDE by oral gavage to these dosages of CPF. The re-
dution of forebrain AChE activity was similar between males and females on PND 4, 7, and 12 with inhibition levels of 25-29%, 33-36%, and 12-15% for 1.5 mg/kg C2P and 41-46%, 44-48%, and 18% for 3.0 mg/kg C2P, respectively. These data suggest that following repeated C2P exposure, male and female rat pups respond similarly.

1532

AGE- AND TIME-DEPENDENT EFFECTS OF EQUI-TOKOUS DOSAGES OF PARATHION OR CHLORPYRIFOS ON HIGH AFFINITY CHOLINE UPTAKE IN RAT Cortex AND STRIATUM.

K.J. Oliver and C. N. Pope. Dept Physiological Sciences, Oklahoma State University. Stillwater, OK.

High affinity choline uptake (HACU) in brain is altered in adult rats after exposure to some organophosphorous (OP) toxicants. Immature rats are more sensitive than adults to the toxicity of OP insecticides parathion and chlorpyrifos, but little is known regarding age-related effects on HACU. AChE and HACU in cortex and striatum of neonatal (7 days), juvenile (21 days) and adult (90 days) rats (n=8-15; treatment group) were evaluated at 4, 24 or 96 hr after equitoxic exposures to parathion (LD10 = 0.81, 1.5 and 6.6 mg/kg, po) or chlorpyrifos (LD10 = 15, 47 and 136 mg/kg, po). HACU was studied by incubation of synaptosomes with [3H]choline in subsequent separation of incorporated and unincorporated label by rapid filtration. Parathion elicited relatively similar levels of peak ChE inhibition in all age groups (64-82% at 4 hr after dosing). Chlorpyrifos also elicited similar age-related reductions in ChE activity (81-91%), but peak inhibition was earlier in neonates and juveniles (4 hrs) than in adults (24 hrs). HACU was increased after parathion exposure, with maximal increases occurring at 4 hrs after exposure in neonatal striatum (52%), juvenile cortex (19%) and striatum (23%), and at 96 hrs after treatment in adult cortex (41%) and striatum (36%). In contrast, HACU was generally reduced by chlorpyrifos, with maximum reduction at 4 hrs after dosing in neonates (43%), 24 hrs in juveniles (22%) and 96 hrs in adults (18%). Interestingly, HACU was increased (15%) in neonatal striatum at 96 hrs after chlorpyrifos. Increased uptake of choline could lead to higher acetylcholine synthesis and amplification of the toxic consequences of parathion-induced acetylcholinesterase inhibition. Alternatively, a reduction in HACU following chlorpyrifos could potentially limit the amount of ChE released into the synapse and thereby reduce cholinergic toxicity. Changes in HACU may contribute to OP toxicant- selective and age-related toxicity following AChE inhibition by these two insecticides. (Supported by STAR grant GR825811 from USEPA).

1533

COMPARATIVE TOXICITY OF ORAL CHLORPYRIFOS EXPOSURES IN ADULT AND AGED RATS.

S. Karanth, K. Oliver and C. N. Pope. Physiological Sciences, Oklahoma State University. Stillwater, OK.

Chlorpyrifos (CPF) is a widely used organophosphorous insecticide that elicits toxicity through inhibition of acetylcholinesterase. A number of studies have reported that CPF is more toxic to immature animals and that aged rats (15 and 24 months of age) are not differentially sensitive to subchronically administered CPF. We evaluated the relative sensitivity in adult (3 months) and aged (15 months) rats to oral CPF exposures. Adult (3 months of age) and aged (15 months of age) Sprague-Dawley rats (n=5) were challenged with an effective but sublethal dosage (LD1) previously determined in adult rats (i.e., 80 mg/kg, po in peanut oil). Adult rats showed no signs of functional toxicity while aged rats exhibited moderate to severe signs (principally autonomic) and 2/5 lethality. Additional groups (n=5) were treated daily with CPF (0, 1, 3 or 10 mg/kg, po) for 14 days and sacrificed for neurochemical measurements 4 hrs after the final dosing. None of the adult or aged rats exhibited functional signs of toxicity throughout the dosing period. Blood cholinesterase inhibition was greater in aged rats (71%) than in adults (55%) at 1 mg/kg/day group. Diaphragm ChE was significantly increased (16-25%) in adult rats at 1 and 3 mg/kg/day while in aged rats there was a dose-related reduction in activity (15-40%). Hiatal cholinesterase was significantly greater in aged rats (81%) compared to adults (39%) at 3 mg/kg/day, whereas at 10 mg/kg/day, ChE inhibition was similar in both groups (86-92%). These results suggest that aged rats may be more sensitive than adults to both acute and repeated oral CPF exposure. (Supported by grant ES09119 from NIEHS).

1534

CHRONIC DIETARY EXPOSURE WITH INTERMITTENT SPIKE DOES OF CHLORPYRIFOS FAILS TO ALTER FLASH- OR PATTERN REVERSAL- EVOKED POTENTIALS IN RATS.


Human exposure to pesticides is often characterized by chronic low level exposure with intermittent spiked higher exposures. Visual disturbances are often reported following exposure to xenobiotics, and cholinesterase-inhibiting compounds have been reported to alter visual function. This study examined the effects of chronic (1 year) dietary exposure (0, 1, or 5 mg/kg/day) to chlorpyrifos in male Long Evans rats (100-110 days old at study initiation). These doses were chosen to produce minimal and approximately 30% inhibition of brain cholinesterase activity, respectively. In addition to dietary exposure, half of the animals received an oral bolus of 45 mg/kg chlorpyrifos (in corn oil) once per week (n=16-18 rats/treatment). Subjects were weighed maintained at 350g throughout the study. After the final spiked exposure, the animals were allowed to recover for about 2.5 months, to only irreversible effects would be examined. Subjects were surgically implanted with screw electrodes over the visual cortex and allowed to recover for at least one week. Unanesthetized animals were placed in a restrainer and presented with a grating-based visual stimulus consisting of a bar pattern that was flashed or moved against a stationary background with or without the presence of oral spike doses did not produce changes in either flash- or pattern reversal-evoked potentials. The evoked potentials did show the expected intensity and spatial frequency dependent changes, showing that the animal's responses were under stimulus control. Thus, chronic exposure to chlorpyrifos did not appear to alter visual responses at the level of the visual cortex in adult animals. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1535

PATTERN OF CHOLINESTERASE INHIBITION IN ADULT, MALE RATS CHRONICALLY EXPOSED TO DIETARY CHLORPYRIFOS.


Very little is known about the effects of chronic exposure to relatively low levels of anticholinesterase insecticides or how the effects of chronic exposure compare to higher, intermittent exposure of the same compound for the same duration. To that end, we exposed adult male rats to the anticholinesterase insecticide, chlorpyrifos (CPF) for one year at three levels of dietary exposure: 0, 1, or 5 mg/kg/day. In addition, those groups also received a bolus dosage of CPF (i.e., "spiked" animals; 60 mg/kg intraperitoneal and 45 mg/kg/day in corn oil every other two months; therefore, there were 6 dosage groups, n=5/group). Clinical, neuronal and behavioral effects were examined during dosing (at 6 or 12 months) and also 3 months after dosing ceased (i.e. "recovery" animals). Tissues analyzed were retina, whole blood and components, diaphragm, and cortical nervous system (pons). In general, the groups receiving dietary CPF exposure only showed substantial cholinesterase (ChE) inhibition (80%) in the blood at 1 mg/kg/day and no inhibition in retina, pons, or diaphragm; the higher dosage (5 mg/kg) produced more blood inhibition and approximately 50% or less ChE inhibition in the other tissues. One day after receiving the oral, spike dose, all three feeding (i.e., 0, 1 or 5 mg/kg/day) groups showed more than 70% inhibition in each tissue. In the dietary exposure groups (unspiked), the ChE inhibition at 6 months was, in general, not different from the initial inhibition at 12 months, indicating that steady state had been achieved prior to 6 months. Three months after CPF dosing ended, ChE had returned to control levels in all groups. These data indicate that although chronic feeding with or without intermittent spiked dosages with CPF produces substantial ChE inhibition in a dose- and tissue-related manner, ChE activity recovers to normal within 3 months after dosing is ceased. This abstract does not reflect EPA policy.

1536

NEUROBEHAVIORAL EVALUATION OF RATS EXPOSED TO CHLORPYRIFOS VIA CHRONIC DIETARY AND REPEATED HIGH-LEVEL SPIKE EXPOSURE.


This study aimed to model long-term subtoxic human exposure to an organophosphorous pesticide, chlorpyrifos (CPF), and to examine the influence of that exposure on the response to intermittent high-dose acute challenges. Adult Long-Evans male rats were maintained at 350g body weight by limited access to CPF-laced food pellets provided to produce an intake of 0, 1, or 5 mg/kg/day (n=40/group). During the year-long exposure, half of the rats in each feed group received bi-monthly challenges (spikes) of CPF, and the other half were received vehicle. Rats were periodically tested, using a neurobehavioal screening battery and motor activity, to evaluate the magnitude of the acute response (spike days) as well as recovery and ongoing chronic effects (non-spike days). In non-spike rats, the lower feed level produced blood borne cholinesterase inhibition, whereas the higher feed level produced about 50% brain cholinesterase inhibition. Effects of the spikes differed as a function of dietary level for several endpoints (e.g., tremor, lacrimation), and in
1539 RELATIVE INHIBITORY POTENCIES OF CHLORPYRIFOS OXON AND CHLORPYRIFOS METHYL OXON FOR HUMAN ACETYLCHOLINESTERASE (ACHE) AND NEUROPATHY TARGET ESTERASE (NTE).

T. J. Kropp and B. J. Richardson, Toxicology Program, The University of Michigan, Ann Arbor, MI.

The relative inhibitory potency (RIP) of an organophosphorus (OP) inhibitor against ACHE versus NTE may be defined as the ratio, k (ACHE)/k (NTE), where k is the bimolecular rate constant of inhibition for a given inhibitor against each enzyme. RIP values > 1 correlate with the inability of OP inhibitors or their parent compounds to produce OP compound-induced delayed neuropathy (OPIDN) at doses below the LD50. Chlorpyrifos oxon (CPF) has a RIP value > 1 for enzymes from hen brain homogenates and the parent compound, chlorpyrifos (CPF), cannot produce OPIDN in hens at sublethal doses. The present study was carried out to test the hypothesis that the methyl homologue of CPF, chlorpyrifos methyl oxon (CPO), also has a RIP value > 1. Hen brain microsomes were used as the enzyme source and k values (X ± SE) were determined at 37°C for ACHE and NTE (n = 3 and 4 separate experiments, respectively). The k values for compounds against ACHE and NTE were 17.8 ± 0.3 and 0.0894 ± 0.0049 µM⁻¹ min⁻¹, respectively, yielding a RIP value of 179 ± 4. The k values for CPO against ACHE and NTE were 10.9 ± 0.1 and 0.0582 ± 0.0013 µM⁻¹ min⁻¹, respectively, yielding a RIP value of 187 ± 4. The results demonstrate that the RIP values for CPF and CPO are comparable and both are >1, suggesting that neither parent compound, CPF nor chlorpyrifos methyl, could produce OPIDN at sublethal doses. (Supported in part by a contract from Dow AgroSciences.)

1540 IMMUNOLOGIC DETECTION & AFFINITY SELECTION OF ORGANOPHOSPHATE-ACETYLCHOLINESTERASE ADDUCTS USING ACTIVE SITE SPECIFIC ANTIBODIES.

L. E. Sandoval1, K. M. George2, D. H. Williamson3 and C. M. Thompson1,2,3,1 Department of Pharmaceutical Science, University of Montana, Missoula, MT; 2 Center for Environmental Health Sciences, University of Montana, Missoula, MT and 3 Department of Chemistry, University of Montana, Missoula, MT.

Organophosphate (OP) insecticides are rapidly absorbed to quickly infiltrate blood and nervous tissue, resulting in the formation of acetylcholinesterase (ACHE). Exposure to OP compounds, therefore, is expected to correlate with formation of an OP-conjugate as determined by measurement of total erythrocyte and serum CHES activity. This method is both limited by population variation in total CHES levels and the inability to identify the responsible OP compound. It is our hypothesis that OP-ACHES conjugates can be reliably detected by antibodies raised specifically against an active site sequence in both its native and phosphorylated states. Such antibodies would be capable of quantifying OP-modified cholinesterase and capable of identifying the OP compound. We have prepared antibodies specific to the active site sequence of mammalian ACHE in both its native and phosphorylated states. Western Blots were employed to examine binding of a panel of antibodies against the native and OP-modified enzyme. Antibodies raised against the active site domain of ACHE recognize the untreated enzyme but fail to bind to ACHE covalently modified by OP's. In contrast, antibodies raised against the phosphorylated active site bind to PCHE treated ACHE (formation of phosphonate) but do not bind ACHE or ACHE inactivated by a phosphoester group. To confirm selectivity of the custom antibodies, samples of treated and untreated ACHE were tryptophan and resultant peptides isolated by affinity chromatography using the active site antibodies. Peptide samples were further separated by capillary liquid chromatography and sequenced by mass spectrometry. Using a combination of immunological techniques, synthetic chemistry and mass spectrometry this presentation outlines methods to detect, identify and differentiate OP-ACHE adducts.

1554 PROTECTION AGAINST PARATHION TOXICITY BY SINGLE OR REPEATED ADMINISTRATION OF PHYSOSTIGMINE IN RATS.

Y.C. Kim, S.Y. Kim and M.G. Lee, Pharmacy, Seoul National University, Seoul, South Korea.

The protective effects of physostigmine against the toxicity of parathion were examined in male Sprague-Dawley rats. Physostigmine (100 or 1, 000 µg/kg, ip) injected 30 min before decreased the inhibition of acetylcholinesterase (ACHE) activities in brain, lung and blood induced by parathion (2 mg/kg, ip). Physostigmine pretreatments did not affect the total hepatic microsomal cytochrome P450 (CYP) or cytochrome b5 contents. There was no change in p-nitrophenol hydroxylase, p-nitroanisole demethylase or aminopyrine N-demethylase activities in liver of physostigmine-pretreated rats. However, erythromycin N-demethylase activity, a selective CYP3A marker, was significantly inhibited by the carbamate in a dose-dependent manner. Physostigmine also decreased erythromycin N-demethylase activity in brain, Physostigmine did not alter carboxylesterase or paraoxonase activity, indicating that detoxication of parathion was not affected by this carbamate. The pharmacokinetic parameters of parathion (3 mg/kg, iv) were determined after a single dose of physostigmine. In physostigmine-pretreated rats, the area under the curve (AUC) of parathion was significantly greater; clearance (Cl) was lower than control rats. Physostigmine (100 or 1, 000 µg/kg/day, ip) was repeatedly administered to rats for 7 consecutive days. When parathion was given to rats 4 or 24 hr after the final dose of the carbamate, the brain ACHE inhibition was reduced significantly. Repeated administration of physostigmine did not affect thymolyn N-demethylase activity or the pharmacokinetic parameters of parathion. However, there was a significant decrease in specific [TH] QNB binding in striatum and hippocampus. Scatchard analysis revealed a significant decrease in Bmax without a change in KD. The results suggest that the inhibition of CYP3A plays an important role in the decrease of parathion toxicity by an acute dose of physostigmine, but changes in characteristics of muscarinic receptors could account for the protection against parathion toxicity in rats treated with this carbamate repeatedly.

1550 AUTOANTIBODY DETECTION USING ELISA AND WESTERN BLOT IN DELAYED NEUROPATHY INDUCED BY ORGANOPHOSPHATES (OP).

B. Vivekandanand1, C. D. Clevell1, A. P. De Foss2 and H. A. El-Fawal1, 1Pharmacology and Toxicology Laboratory, Mercy College, Dobbs Ferry, NY and 2Environmental Medicine, NYU Medical Center, Tucson, AZ. Sponsor: J. O’Callaghan.

Earlier studies demonstrated the attenuating effects of calcium channel blockers, and calpain inhibitors on the development of OP-induced delayed neuropathy (OPIDN) in hens. This modifying effect was evident due to the attenuation of the calcium-dependent protease calpains activity in brain and nerves. Selective calpain inhibitors 1 (Cal1) and 2 (Cal2) were used to modify the development of OPIDN following a single dose of phenyl saligenin phosphate (PSP, 5 mg/kg, im,
1542 ORGANOPHOSPHATE PESTICIDES DIFFERENTIALLY ALTER AXONAL AND DENDRITIC GROWTH IN CULTURED SYMPATHETIC NEURONS.


1Environmental Health Science, John Hopkins University, Baltimore, MD and 2Biology, Canisius College, Buffalo, NY.

There is increasing evidence that cognitive and behavioral problems may be linked to perinatal exposure to environmental chemicals, such as organophosphate pesticides (OPs). However, the mechanism(s) by which OPs cause changes in neuronal function have yet to be determined. We used primary cultures of sympathetic neurons to investigate the possibility that OPs interfere with morphogenetic events critical to establishing neuronal connectivity, specifically axonal and dendritic outgrowth. When grown in serum-free medium in the absence of glial cells, sympathetic neurons extend a single axon and no dendrites. In contrast, neurons grown in the presence of either glial cells or bone morphogenic proteins (BMPs) extend multiple dendrites. Neurons grown under these conditions were exposed to chlorpyrifos (CPF, 0.1mM to 10 uM) or its oxon metabolite, CPF0 (0.01 nM to 10 nM) for 24 or 72 hr then immunostained for either axon- or dendrite-selective antigens. Axonal and dendritic growth were quantified using Metamorph image analysis software. A 24-hr exposure to either CPF or CPF0 causes a significant inhibition of axonal outgrowth in sympathetic neurons even at the lowest concentrations tested. In contrast, a 72-hr exposure to the same concentration range of these OPs weakly induces dendritic growth in the absence of BMPs, and enhances dendritic growth observed in the presence of BMPs. These effects on axonal and dendritic growth occur in the absence of effects on cell viability or acetylcholinesterase enzymatic activity, which indicates that OPs may exert specific and differential effects on axonal and dendritic growth. These data support the hypothesis that OPs disrupt patterns of neuronal connectivity in the developing brain.

1543 REMEDIATION OF ORGANOPHOSPHORUS NEUROTOXICITY IN SY5Y NEUROBLASTOMA CELLS BY ORGANOPHOSPHORUS HYDROLASE (OPH).


Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

In a previous study, model organophosphorus insecticides (OPs) produced anticholinesterase activity, and mipafos and paraxon modified the expression of the proteins associated with the neuronal cytoskeleton in human SY5Y neuroblasto- maa cells. The patterns of protein modification were different between mipafos and paraxon. Numerous approaches have been studied to degrade OP compounds and remediate their toxicity. In the current study, biodegradation of model OP compounds and its efficiency to prevent or reduce neurotoxicity of the compounds were tested with SY5Y cells. OPs pretreated with organophosphorus hydrolase (OPH) did not inhibit acetylcholinesterase (AChE) and neuropathy target esterase (NTE) in SY5Y cells as potently as did buffer-treated OPs. The anti-AChE activity of paraxon (maximum 3 µM) and anti-NTE activity of mipafos (250 µM) in SY5Y cells were completely prevented by degradation with OPH. Mipafos (0µM) produced significant modifications in expression of MAP2 and NT200 proteins in SY5Y cells, while equimolar paraxon did not. The reaction of NGE-differentiating SY5Y cells to mipafos on protein modulation was dependent on the stage of differentiation at which the OP compound was applied. Neurofilament 200 protein in SY5Y cells exposed to the OPH-treated mipafos containing about 4 µM of mipafos remained after biodegradation when given during the early stages of cell differentiation, but upregulated when given at a late stage.

Biodegradation of mipafos with OPH significantly reduced neurotoxicological stress on the modulation of these neuroskelton protein biomarkers. These results support the conclusion that OPH can be used to biodegrade OPs and remediate their neurotoxic effects in vitro.

1544 EFFECTS OF NEUROPATHY-INDUCING ORGANOPHOSPHORUS (OP) COMPOUNDS ON MITOCHONDRIAL ATP PRODUCTION IN PRIMARY DORSAL ROOT GANGLIA CELL CULTURES.

C. Massicotte, C.J. van der Schyf, B.S. Jorner and M. Ehrich. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Cultures of dorsal root ganglia (DRG) can achieve neuronal maturation with axons. They have not, however, previously been used to investigate metabolic events that occur following exposure to neuropathy-inducing organophosphorus (OP) esters. Recent studies in other systems demonstrated in vitro alterations of ATP concentrations and changes in mitochondrial transmembrane potential following exposure to neuropathy-inducing OP compounds, suggesting that mitochondrial dysfunction occurs. We report an investigation using chick embryo DRG cultures to evaluate effects on the mitochondrial respiratory chain associated with exposure to these toxicants. This approach uses an in vitro neuronal system from the animal species (chicken) that provides the USEPA-approved animal model for OP-induced delayed neuropathy (OPIDN). DRG were obtained from 9-10 day old chick embryos and grown for 14 days in minimal essential media (MEM) supplemented with bovine and human placental serum and growth factors. Cultures were then treated with 1 µM phenylisothiocyanate phosphate (MIP), mipafos, paraxon, or the DMSO vehicle. Mipafos (100 µM) readily elicits OPIDN in hen; paraxon does not. [314] in situ evaluation of ATP production measured by bioluminescence assay in neurons treated with MIP and mipafos, but not paraxon, showed significantly (p<0.05) decreased ATP concentrations. Values (pmol/cell) mean ± SEM, n=6 were 307 ± 8 in control cultures, and 278 ± 17, 189 ± 5 and 256 ± 14 in paraxon, MIP and mipafos-treated cells, respectively. This low energy status was present in several levels of the mitochondrial respiratory chain, including complexes I, III and IV, although complex I was the most severely affected (~40% of control following mipafos and OP treatment, p<0.05; paraxon had no significant effect). We conclude that mitochondria are a primary target for neuropathy-inducing OP compounds, resulting in depletin in ATP concentrations.

1545 PHENYL SALIGENIN PHOSPHATE INDUCED SPINAL CORD GENE EXPRESSION CHANGES IN ADULT GALLUS GALLUS.

J. Fox, M. Ehrich, S. P. Hancock and B. S. Jorner. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Adult hens (Gallus gallus) exposed to a single dose of phenyl saligenin phosphate (PSP) develop delayed onset central-peripheral distal axonopathy. Mechanisms of this axonopathy are thought to be initiated by inhibition and aging of the carboxylesterase and glutathione-S-transferase (PTE). However, little is known about how this inhibition of PTEs affects aging and onset of neurologic deficits. We hypothesized that spinal cord gene expression changes occur in the spinal cord in response to PSP. Adult hens (Gallus gallus) were treated with 50 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg of PSP, and were sacrificed 48 hours post-treatment. The spinal cord was removed and homogenized, and RNA was extracted using the TRIzol method. RNA samples were reverse-transcribed to cDNA, and real-time PCR was performed using SYBR Green dye and the LightCycler system to amplify specific cDNA sequences. The results showed that the expression of several genes, including those encoding for the protein-tyrosine phosphatase (PTP), were altered in the spinal cord of PSP-treated hens. These results suggest that PSP-induced spinal cord gene expression changes may contribute to the development of PSP-induced axonopathy and provide important insights into the mechanisms underlying this disease.
OPIDN in White Leghorn hens as determined by staining of brain and spinal cord with hematoxylin and eosin (H&E) and a Fluoro-Jade, fluorescent stain for degenerating neurons and their processes. Hens were injected with single doses of the Type I neurotoxicant phenyl saligenin phosphate (PSp: 2.5 mg/kg/imb), the Type II neurotoxicant beta phenyl toluene (BP: 750, 300 mg/kg/sc), or the vehicle dimethyl sulfoxide (DMSO, 0.5 ml/kg, im or sc). They were evaluated daily for clinical signs of neural dysfunction and sacrificed after one, two, and three weeks. PSp- and TPI-injected hens displayed progressive neurological dysfunction beginning about one week post-administration. The brain and lumbar levels of spinal cord were prepared for microscopic study, as noted above. Samples of the nerve to the biventer cervicis muscle were also prepared for light microscopic examination. H&E stained sections from PSp-dosed hens revealed degenerating myelinated fibers in regions such as long spinal cord and medullary tracts. TPI-dosed hens had these along with neuronal necrosis in spinal, brainstem and deep cerebellar regions. Staining with Fluoro-Jade revealed more profound and extensive time-dependent PSP- and TPI-induced degeneration of nerve fibers, terminals, and cell bodies (for TPI) in spinal, brainstem and cerebellar regions. Both compounds elicited peripheral nerve axonopathy progressing to Wallerian-like degeneration of a similar (severe) intensity. This study demonstrates the utility of Fluoro-Jade staining to reveal the extent of neuronal degeneration in OPIDN, and the presence of a peripheral neuropathy in the Type II form.

1547
A QUANTITATIVE HISTOCHEMISTRY TECHNIQUE FOR MEASURING REGIONAL DISTRIBUTION OF ACETYLCOLINESTERASE AND BUTYRYLCOLINESTERASE IN THE BRAIN USING DIGITAL SCANNING DENSITOMETRY.
T. Ma, Z. Cal, S. E. Wellman and J. K. Ho, Pharmacology & Toxicology, University of Mississippi Medical Center, Jackson, MS.

Studies of brain acetylcholinesterase (ACHE) are traditionally based on biochemical assays, immunoreactivity, and histochemistry. Conventional histochemistry yields rich morphological data from tissue sections but yields quantitative results only with great difficulty. Several histochemical methods developed in recent years, including microdensitometry, microphotometry and video-based histochemistry, are effective in quantitative and detailed study of ACHE in tissue sections. However, they are usually time-consuming. As we report here, we adapted digital scanning densitometry to quantitate ACHE histochemical staining in brain sections. The ACHE and butyrylcholinesterase (BuCHE), as measured by the method, were heterogeneously distributed throughout the brain, results that are consistent with those obtained by biochemical methods. The staining intensity is dependent on section thickness, substrate concentration, and reaction time. The cholinesterase inhibitor methyl paraxanox significantly decreased ACHE staining intensity. Furthermore, data acquired from densitometry are similar to those obtained by video-based microscopy or by spectrophotometry. The advantage of the digitometric measurements compared to other quantitative histochemical methods is that it is very rapid, while collecting data that are equivalent in quality. Because the digital scanning densitometers provide high quality and sensitive imaging, wide dynamic ranges, and convenient image analysis software, they are very useful tools in quantitative histochemistry.

1548
MONITORING BLOOD ACETYLCOLINESTERASE ACTIVITIES IN RATS GIVEN MULTIPLE DOSES OF ORGANOPHOSPHORUS COMPOUNDS.
M. Ehrich, K. Furhman, L. Correll, S. Hancock, L. Floiry and B. S. Jarner, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Acetylcholinesterase (ACHE) inhibitions in whole blood and brain were used to examine interactions of organophosphorus (OP) compounds in adult male Long-Evans rats given chlorpyrifos (60 mg/kg sc) followed in one week by oral tri-ortho-tolyl phosphate (TOTP: 75, 150 or 300 mg/kg) every other day for 2 weeks before sacrifice. ACHE inhibition was monitored in blood 48 hr after dosing with chlorpyrifos, 5 days after initiating TOTP treatment, and on the day of sacrifice. At sacrifice, ACHE activities in hippocampus, cortex, basal forebrain and caudate putamen of rats given only chlorpyrifos were 45% ± 5% (mean ± SE, n = 6), 33% ± 5, 43% ± 7, and 39% ± 7 of control, respectively. ACHE activities in these brain regions after treatment with chlorpyrifos + 300 mg/kg TOTP were 28% ± 5, 18% ± 2, 25% ± 5, and 18% ± 2, respectively. ACHE activities after chlorpyrifos + 300 mg/kg TOTP were in the range of 11% - 18% of control in hippocampus, cortex, caudate putamen and basal forebrain. In the cortex, chlorpyrifos caused blood ACHE activities to drop to 45% ± 2 of control 48 hr after dosing with activity remaining this depressed at the time of second bleeding, but returning to 85% ± 12 of control (considerably higher than in any brain region) at time of sacrifice. Following chlorpyrifos and 5 days and 2 weeks of 300 mg/kg TOTP, blood ACHE activities were 41% ± 11 and 31% ± 7 of control, respectively. Lower doses of TOTP caused lesser ACHE inhibitions in both blood and brain. Corticosterone in drinking water did not alter blood ACHE activities. Therefore, blood ACHE activities provided dose-response data, although activities were significantly higher than in brain of rats given one OP compound (chlorpyrifos) weeks earlier. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)

1549
BEHAVIORAL CHANGES IN RATS GIVEN TRIOPTHROTOXYL PHOSPHATE (TOTP) AFTER CHLORPYRIFOS AND CORTICOSTERONE: LACK OF DOSE-RELATED CORRELATION WITH EFFECTS ON GLUTATHIONE PEROXIDASE, SUPEROXIDE DISMUTASE AND GMP.
L. Correll, L. Floiry, J. Hinckley, S. Hancock, D. Ward, M. Ehrich and B. S. Jarner, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Motor activity, a Functional Observational Battery (FOB), and passive avoidance were used to evaluate rats given tri-ortho-tolyl phosphate (TOTP) 75, 150 or 300 mg/kg, p.o. every other day for 2 weeks, beginning 1 week after a single administration of chlorpyrifos (60 mg/kg sc). In addition, half of the rats in each group were given corticosterone (400 mg/ml) in drinking water beginning 7 days before administration of chlorpyrifos. The behavioral endpoints were measured the day before dosing with chlorpyrifos, and 2 and 3 weeks later. Chlorpyrifos administration resulted in decreased beam breaks (an indicator of motor activity) in the presence and absence of corticosterone (p<0.007). TOTP decreased ambulation after 2 weeks of dosing. Chlorpyrifos exaggerated effects on motor activity and on limb grip strength caused by corticosterone treatment alone. No treatment affected passive avoidance. Biochemical studies at terminal sacrifice revealed that neither corticosterone, TOTP or chlorpyrifos had effects on glutathione peroxidase (GPx) or superoxide dismutase (SOD) activities in the cerebral cortex or hippocampus. Gli fibrillary acidic protein (GFAP) was elevated by all treatments (corticosterone, TOTP, chlorpyrifos) in cerebral cortex, but effects were not dependent on dose of TOTP. These results indicate that each treatment had effects on behavior, but they were not dependent on alteration of enzymes indicative of oxidative stress (GPx, SOD). GFAP however, appeared useful for identification of rats exposed either to corticosterone or organophosphates. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)

1550
THE ORGANOPHOSPHORUS NEUROTOXICANT TRIORTHOTOLYX PHOSPHATE CAUSES LIVER DYSFUNCTION IN RATS.
K. Zimmerman, M. Ehrich and B. S. Jarner, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

The interactions of stress and exposure to neurotoxic chemicals are a matter of considerable interest in toxicology. In a study designed to evaluate these, young adult male Long-Evans rats were administered the following (alone or in various combinations): 400 mg/ml corticosterone in the drinking water (the stress model) from days 1-63; 60 mg/kg sc chlorpyrifos on days 7 and 42; tri-ortho-tolyl phosphate (TOTP: 75, 150 or 300 mg/kg) by gavage every other day from days 14-26 and 49-56. This portion of the study evaluated clinical chemistry and hematologic findings in the blood collected at sacrifice (day 63). Corticosterone exposure alone or in combination with chlorpyrifos produced some significant variation of analytes. However, a significant alteration in cholesterol concentration (mean 114 ± 11 mg/dL compared to 85 mg/dL in controls, p = 0.017) occurred with TOTP exposure at 150 mg/kg. Exposure to TOTP at 300 mg/kg produced alterations in cholesterol (mean 125 ± 11 mg/dL, p = 0.003) and alanine aminotransferase (ALT) activity (mean 142 U/L compared to 52 U/L in controls, p = 0.041). TOTP at 75 and 150 mg/kg combined with corticosterone and chlorpyrifos individually or in combination produced significant alterations in cholesterol concentrations (mean range 127 - 171 mg/dL, p = 0.028 - 0.046). In addition, TOTP at 300 mg/kg, combined as described above, resulted in alteration in albumin concentration (mean 2.8 ± 0.5 mg/dL, p = 0.055), albumin globulin ratio (mean 0.73 ± 0.055), urea nitrogen concentration (mean 13.3 mg/dL, p = 0.001), cholesterol (mean 131 mg/dL, p = 0.030), and ALT activity (mean 153 U/L, p = 0.032). Therefore, multiple administration of TOTP to Long-Evans male rats at doses of 75 mg/kg and greater appears to alter hepatic metabolic parameters. TOTP at 300 mg/kg resulted in reduced hepatic function. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)
Hippocampal slice cultures were used to examine the action of the organophosphate agent soman (GD). Soman is known to inhibit acetylcholinesterase, causing accumulation of ACh, but it also appears to increase glutamate release to promote seizure-related damage. In the present study, the effects of low concentrations of soman on hippocampal tissue were assessed over an extended series of exposures. Single application was used to induce a period of toxicity until agent hydrolysis, and this was repeated daily for one week. No evidence of degeneration was found after the first day of treatment, however, dose-dependent declines in pre- and postsynaptic proteins occurred after a week. The decline in synapsophyalin was evident at the 100-nM dose of soman, and further reduction was found at 20 μM (ANOVA: p<0.001, F=9.7). Synapsophyalin was reduced throughout the hippocampus, especially in dentate fields of CA1 and in the molecular layer of the dentate gyrus. Synaptic toxicity appears first since overt cellular pathology was not visible in the three subfields. The decline in the postsynaptic GluR1 subunit was only evident at the 20-μM soman dose (p<0.001, F=11.8). Soman also elicited a pronounced increase (p<0.01) in spectrin breakdown mediated by calpain, a protease known to cause neurodegeneration in response to glutamate-induced excitotoxicity. Interestingly, while no degeneration was found after the first day of soman treatment, pathogenetic changes did occur when the soman insult was combined with a trimethyltin (TMT) insult - TMT is known to cause seizures and hippocampal damage. A 1-h TMT exposure did not cause appreciable synaptic deterioration or spectrin breakdown in control slices, however, robust levels of degeneration were evident when such a TMT insult was followed by a single application of soman. Thus, low-level soman exposure causes synaptic damage in the hippocampus and potentiates vulnerability to other types of toxic insults. Supported by US Army Med. Res. grant DAMD17-99-C-0986, and NIH grant 1R43NS38494-01.

M. B. Abu-Douia, A. A. Abdel-Rahman, and A. K. Shetye, Pharmacology and Cancer Biology and Neurosurgery, Duke University Medical Center and VA Medical Center, Durham, NC.

We evaluated the early changes in the adult brain of male Sprague-Dawley rats following single i.n. injection of 1, 0.5, 0.1, and 0.01LD₅₀ of sarin. Twenty four h after treatment, both sarin-and vehicle-treated control animals were processed for: 1) BBB permeability, 2) brain acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) activity, 3) brain m2 muscarinic acetylcholine receptor (m2 mAChR) ligand binding, and 4) histopathological examination of brain. Animals treated with 1 and 0.5 x LD50 exhibited a significant inhibition of BChE activity, while 1 x LD50 caused a significant inhibition of AChE in the cerebellum, brainstem, midbrain, and cerebrum. Ligand binding to m2 mAChR decreased in cerebrum and showed a significant increase in brainstem. Analysis of [3H]hexamethonium iodide uptake demonstrated a significant increase in BBB permeability in animals treated with 1 x LD50. The LD50 dose caused significant decrease in the expression of BBB protein, a diffuse neuronal cell death and a decrease in MAP-2 positive elements within the cerebral cortex and hippocampus, and degeneration of Purkinje cells in the cerebellum. Animals treated with 0.5, 0.1, or 0.01 x LD50 did not exhibit the above changes except in the cerebellum, where animals treated with 0.5 x LD50 showed Purkinje neuron loss. Thus, the early brain damage after acute exposure to sarin induces significant injury in many regions of the adult brain by 24 h after exposure. The early neuropathological changes observed after a single 1 x LD50 sarin could lead to a profound long-term neurodegeneration in many regions of the brain, and neurobehavioural abnormalities. Supported, in part, by the US Army Medical Research and Materiel Command under contract DAMD 17-98-C-0827.

S. M. Johnston and B. A. Bahr, Dept. of Pharmaceutical Sciences and the Neuroscience Program, University of Connecticut, Storrs, CT.

Low-level soman exposure leads to synaptic damage in the hippocampus and potentiates neuronal vulnerability.

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ACUTE EXPOSURE TO SARIN INCREASES BLOOD-BRAIN BARRIER (BBB) PERMEABILITY AND INDUCES NEUROPATHOLOGICAL CHANGES IN THE RAT BRAIN.


Molinate is a thiocarbamate herbicide used in the rice industry for over 25 years and has been shown to produce testicular toxicity in male rats. It has been proposed that bioactivation of molinate via sulfonation to form the sulfoxide and sulfoxone metabolites capable of protein binding, may be involved in molinate-induced testicular toxicity. Based on previous in vitro studies, carbamylation of an active site serine residue in Hydrolase A has been proposed to be the mechanism responsible for the observed testicular toxicity. The experiments presented here utilize molinate to characterize covalent protein modifications produced in vivo by molinate in rats dosed intraperitoneally as a function of exposure duration. Examination of globin from molinate-treated rats by HPLC demonstrated a new peak in the isolated samples that when collected and analyzed using MALDI-TOF MS, revealed a 126 Da increase in mass relative to the native β3 chain. Digestion of the globin using Glu-C and analysis by MALDI-TOF MS revealed two modified peptide fragments at m/z 2743 and 4985 consistent with a 126 Da increase to peptide fragments [122-146] and [102-146] in the unmodified β2 and β3 chains of globin. Selected reaction monitoring LC/MS/MS was used to detect the presence of S-hexahydr-1H-azepine-1-carboxyl cyanide (HAC-Cys) in the globin hydrosylates isolated from the molinate-treated rats and to confirm the structure of the modification. These experiments demonstrate the ability of molinate to covalently modify proteins in vivo in a dose dependent manner via carbamylation at Cys-125, similar to the modification identified in rat and N-N-diethylthiocarbamate. The ability of molinate to covalently modify cysteine residues provides a potential mechanism to account for enzyme inhibition following molinate exposure and suggests that enzymes with cysteine residues in their active site may be inhibited by molinate. These results may also extend to other structurally related pesticides.

1554

CHARACTERIZATION OF A S-HEXAHYDRO-1H- AZEPINE-1-CARBOXYL ADDUCT PRODUCED BY MOLINATE ON RAT HEMOGLOBIN β2 AND β3 CHAINS IN VIVO.

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GLUTATHIONE DEPLETION IN RAT PRIMARY HEPATOCYTES AND LIVER BY N-METHYLDITHIOCARBAMATE (NMDC) AND N-N-DIMETHYLDITHIOCARBAMATE (DMDC).

R. W. Thompson and W. M. Valentine, Pathology, Vanderbilt University Medical Center, Nashville, TN.

Several decomposition and metabolic pathways are shared by the commercially used monomethyl and dialkyl dithiocarbamates that can alter intracellular thiol status. This investigation examines the role of glutathione in the cytotoxicity of dithiocarbamates and investigates the primary mechanisms responsible for glutathione depletion by NMDC and DMDC in cultured primary hepatocytes and perfused rat liver. Because monomethyl dithiocarbamates can acylate intracellular thiols via production of an isothiocyanate (ITC) intermediate, the contribution of ITC to the cytotoxicity of NMDC was also determined. Viability was assessed in hepatocyte cultures by LDH release while reduced, oxidized and S-methylthiocarbamoyl glutathione (GSMTC) levels were determined using HPLC and LC/MS/MS in both cultured hepatocytes and bile samples. The cytotoxicity for both NMDC and DMDC was inversely correlated with intracellular levels of GSH. Although hepatocytes exposed to either NMDC or DMDC had diminished intracellular levels of GSH, DMDC appeared to oxidize GSH to GSSG whereas NMDC exposure resulted in generation of GSMTC. In bile samples, GSMTC was also detected.
within 5 minutes of infusion of NMDC into the liver, however, GSSG was not elevated upon DMDC exposure. These results demonstrate that distinct processes involved in lowering glutathione levels in hepatocytes following incubation with NMDC or DMDC. For the monomethyl dihydroxyacetone generation of the reactive ITC followed by acylation of cellular thiols appears to play a significant role. The rapid appearance of GMSMT in bile samples from NMDC infused livers indicate that enzymatic systems may be involved. In contrast, dialyzed dihydroxycarbame altered intracellular thiol status in cultured hepatocytes by oxidation of GSH but a similar observation was not obtained in the perfused liver experiments. It is anticipated that the results obtained for these two dihydroxycarbames may be applicable to other monooxal and dialyzed dihydroxycarbames.

1556 ETHYLENE-BIS-DIHYDROXICARBAMATES CAN INHIBIT ISOLATED BRAIN AND LIVER MITOCHONDRIA.
V. A. Fieser et al., V. Amarnath and T. J. Moniz,
Center for Molecular Neurosciences, Vanderbilt University, Nashville, TN, Center for Molecular Neurosciences, Vanderbilt University, Nashville, TN 37232, and Center for Molecular Neurosciences, Vanderbilt University, Nashville, TN.

Dihydroxycarbame (DTC) pesticides, such as maneb (manganese ethylene-bis-dihydroxycarbame, EBDTC), have been reported in the literature to cause parkinsonism in certain individuals. Recent data from other labs have also demonstrated that mice treated with maneb show neurodegeneration, which is specific to the striatum. Our hypothesis is that maneb can initiate this neurodegeneration through the production of reactive oxygen species. One mechanism by which maneb could facilitate the formation of free radicals is through inhibition of mitochondrial respiration. Brain and liver mitochondria, isolated from C57 BI/6 male mice, were treated with various concentrations of DTC compounds in oxygen consumption chambers equipped with oxygen electrodes. Exposure was done for 2 hours. EBDTC and diethylthiodihydroxycarbame (DEDTC), were used in order to determine the relative importance of the organic or metal constituent in inhibiting respiration. Maneb, zineb (Zn(EBDTC)), (NH)EBDTC, and Mn(DEDTC) were all shown to inhibit mitochondria in the mmoL/mg protein range when glutamate/malate was used as a substrate for complex I. Furthermore, brain mitochondria were more severely inhibited than liver mitochondria, suggesting a mechanism for brain selectivity. The potency of compounds for brain mitochondria was Mn(DEDTC) > EBDTC > maneb = zineb. For liver mitochondria the potency was zineb > Mn(DEDTC) > EBDTC = maneb. This data suggests that, for mitochondrial inhibition, the organic moiety plays a more significant role than the metal in reducing mitochondrial respiration.

1557 MANEB AND PARAOXAT EFFECTS ON ANTIOXIDANT SYSTEMS IN PC12 CELLS.
D. Lee, D. A. Corp-Slecha and J. A. Spanakos,
Environmental Medicine, University of Rochester, Rochester, NY.

Environmental chemical exposures are potential risk factors for Parkinson's disease (PD). Recently, combined paraquat (PQ) and maneb (MB) exposure has been shown to produce synergistic effects in the nigrostriatal dopamine system in vivo. Although PQ is a redox cycling compound, the mechanisms by which MB or combined agrochemical exposures produce neurotoxicity are unresolved. In this study, PC12 cells were used as a model to assess the effects of MB and evaluate mechanisms of neurotoxicity following combined agrochemical exposure. Cultures were exposued to various concentrations of MB, PQ, and combined PQ+MB for 6-48 h then assayed for cell viability by measuring lactate dehydrogenase (LDH) release into the culture media. LDH levels did not differ between control and treated cells until 48 hours, when there was a slight concentration-dependent elevation in both the MB and combined treatment groups. To assess intracellular redox status, total glutathione (GSH) was enzymatically measured following treatments. Total intracellular GSH increased between 6 h and 24 h following MB treatment, with maximal levels reaching 22% of control. By 48 h, GSH levels remained elevated in cells treated with the highest concentration of MB alone. In combined MB+PQ treatment groups, GSH levels declined by 30% in the lower dose combination groups after 24 h, compared to MB treatment alone. Higher concentration combinations resulted in a more robust elevation of intracellular GSH, which increased with treatment duration. To further evaluate antioxidant defense systems, Cu/Zn- and Mn-SOD protein levels were monitored by immunoblot analysis. At 6 h, Cu/Zn-SOD levels were elevated following treatment with both low and high dose combinations compared to control. A separate set of experiments showed that PQ disrupts cellular antioxidant defense systems, ultimately resulting in altered cellular homeostasis and function. Supported by NIH ES10791 and ES1247.

1558 GENDER DIFFERENCES ASSOCIATED WITH DEVELOPMENTAL AND ADULT EXPOSURE TO PARAGUAT AND MANEB.
M. Thiruchelvam, E. K. Richfield, B. M. Goodman and D. A. Corp-Slecha,
Department of Environmental Medicine, University of Rochester School of Medicine & Dentistry, Rochester, NY.

Parkinson disease (PD), an aging neurological disorder, for unknown reasons is more prevalent in males than females. While the etiology of PD remains unclear, environmental factors are strongly implicated. We have previously demonstrated that combined exposure to paraquat (PQ) and maneb (MB) produces an early irreversibly PD phenotype, including potentiated reductions in locomotor activity and loss of nigrostriatal dopamine neurons in adult male mice. To determine whether gender effects are observed with this model, male and female C57BL/6 mice were exposed developmentally to saline, 0.5 mg/kg PQ, 1 mg/kg MB or PQ+MB from postnatal (PD) day 5-19. A subset of these mice was re-challenged as adults (AD) with saline, 10 mg/kg PQ, 30 mg/kg MB or PQ+MB. A separate group was exposed only as adults. PN exposure to PQ+MB decreased locomotor activity similarly in males and females at 6 weeks of age. By 6 months of age, PQ+MB treated males showed a progressive decrease in activity compared to 6 weeks of age, whereas the females showed a progressive recovery, with activity levels returning to control levels at this time-point. Consistent with this, PN only exposure significantly decreased striatal DA, DOPAC and HVA levels in males and females exposed to PQ alone and PQ+MB, with greater decreases observed in males. PN + AD exposure significantly decreased striatal DA, DOPAC and HVA in all male treatment groups to a greater extent compared to PN alone exposure. In contrast, DA and melatonin levels in PN + AD-fed males treated with PQ and MB alone and in combination were not significantly reduced. The females showed a significantly greater decrease compared to the males. Further, females exhibited full recovery, but males only partial recovery of locomotor activity following AD only exposure to PQ+MB. These data suggest protective mechanisms in females that may be provided by estrogen, which has repeatedly been demonstrated to be a neuroprotective agent against dopaminergic neurotoxins.

1559 FIXED RATIO (FR) SCHEDULE-CONTROLLED BEHAVIOR IN RATS FOLLOWING EXPOSURES TO PARAGUAT AND MANEB.
M. R. Bauter, D. A. Corp-Slecha, M. J. Thiruchelvam, B. M. Goodman and S. Y. Stevens,
Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY.

Previous studies have shown that combined exposure to paraquat (PQ) and maneb (MB) produce potentiated, selective, and irreversible neurotoxicity to the nigrostriatal dopamine system in C57BL/6 mice. This includes sustained reductions in locomotor activity, loss of DA and of DA neurons in the substantia nigra and serves as a model of early environmental Parkinsonism (PD). To determine the specificity of these effects, and attempt to develop a comparable rat model of PD, male Long Evans rats were trained to lever press for food reinforcement on a FR schedule. Reductions in response rates on FR schedules have been related to DA loss in the nigrostriatal DA system as induced by the neurotoxicant 6-OHDA. Thus, it was utilized here as a means of preclinical detection of DA loss. The FR requirement was increased to an FR 40 and continued until a stable baseline was attained. Rats were then treated twice a week for 3 weeks with saline, 10 mg/kg PQ, 30 mg/kg MB, or PQ+MB for a total of 5 doses. PQ and MB administered alone produced marginal decreases in overall response rates (OR) and run rates (RR) compared to control on the FR schedule. Administration of combined PQ+MB resulted in decreases in both measures that appeared to increase in magnitude with successive doses. For 24 hr post-dosing, OR and RR in the PQ or MB alone groups had fully recovered to control levels, whereas rates in the combined PQ+MB group showed substantial reductions and only returned to control levels 48 hr post-dosing. This 24 hr post-dosing reduction in FR rates in the PQ+MB group also seemed to increase with successive treatments. Rats are known to be resistant to MPTP, the commonly used neurotoxicant model of PD. Unlike MPTP, PQ+MB seem to exert greater effects in the rat than were observed in mice where total regimens of 12 injections using these same doses of PQ+MB were administered without any signs of overt toxicity. Supported by ES10791, ES1247, & ES07026.

1560 PARAGUAT TOXICITY IS NOT MEDIATED BY THE DOPAMINE TRANSPORTER.
Y. Quan and G. W. Miller,
Division of Toxicology and Pharmacology, University of Texas at Austin, Austin, TX.

Parkinson's disease is characterized by the loss of nigrostriatal dopaminergic neurons and the resultant loss of dopamine input to the striatum is thought to cause the clinical symptoms. Pesticide exposure is considered to be a risk factor for
Parkinson's disease. The herbicide parquat has been suggested as a candidate environmental chemical risk factor due to its structural similarity to 1-methyl-4-phenylpyridinium (MPP+), the active component of dopaminergic neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydrodipyrindine (MTPP). In addition, mice treated with combined parquat and maneb exhibit the toxicity in the nigrostriatal dopamine system. Our lab has generated an inducible system for dopamine transporter (DAT) in SK-N-MC neuroblastoma cell line. Using this system, we have already shown that MPP+ toxicity corresponded with DAT expression. To determine the role of DAT in parquat neuron toxicity, we tested the effect of parquat in the DAT-mediated dopamine uptake. Only the 10mM parquat partially inhibited the dopamine uptake (~20%). Furthermore, we treated the inducible DAT cell line with serial concentrations of parquat (0, 0.05, 0.1, 0.25, 0.5, 0.75, 1 mM). The toxicity corresponded with the concentration used and was independent of the DAT expression level while the MPP+ toxicity corresponded to the DAT expression level. These findings indicate that while parquat and MPP+ have similar chemical structures, parquat is not transported by DAT. Future studies should be aimed at identifying the mechanism by which parquat targets the dopamine neuron. (Supported by NIH ES-09248, ES-07786, and NS-37951)

1561 MODULATION OF URINE STRIATAL Dopamine TRANSPORTER EXPRESSION BY THE PYRETHEROID INSECTICIDE PERMETHRIN. J. S. Gillette and J. R. Bloomquist. Entomology, Virginia Polytechnic and State University, Blacksburg, VA.

The pyrethroid insecticide permethrin may play a role in the development of Parkinson's disease as a consequence of Gulf War Syndrome. Previous work in our laboratory has shown that an i.p. dose of 1.5 mg/kg permethrin in the C57BL/6 mouse causes a significant increase in striatal dopamine uptake to 125% of control values. Recently, we have dosed C57 mice with either 0.8 or 1.5 mg/kg permethrin (3 i.p. injections over 2 weeks). To assess reversibility of effects on striatal dopamine transporter (DAT), a portion of the mice were sacrificed on the day following the last dose (t=0), while a second group was sacrificed 2 weeks post last treatment (t=2), and a third group 4 weeks post last treatment (t=4). At t=0, DAT protein as assayed by Western blotting was increased 119% and 107% in the 0.8 and 1.5 mg/kg group, respectively, over that of control mice. DAT binding was analyzed by incubating the ligand [3H]GBR 12935 with striatal synaptosomes from control and treated mice. [3H]GBR 12935 binding was increased 156% and 118% in mice treated with 0.8 and 1.5 mg/kg permethrin, respectively. Preliminary evidence suggests that this effect is persistent, since DAT protein was likewise elevated in the t=2 group of mice. Due to its effects on mouse DAT, the ability of permethrin to synergize the effects of the neurotoxin MPTP was ascertained. MPTP (50 mg/kg) was administered to C57BL/6 mice on day 1 of the treatment, followed the next day by administration of various doses of permethrin from 0.8 mg/kg to 200 mg/kg. Striatal dopamine and its metabolite, DOPAC, were determined by HPLC-ECD. Only the highest dose of permethrin (200 mg/kg) in combination with MPTP was able to deplete dopamine significantly greater than MPTP alone. However, DOPAC levels were significantly elevated in mice above 50 mg/kg permethrin, suggesting an increase in dopamine turnover. Taken together, these data suggest that insecticides such as permethrin may have subtle effects on the dopaminergic system, and may be involved in the etiology of Parkinson's disease.

1562 DECREASE OF DOPAMINE LEVELS AFTER PYRETHEROID TREATMENT. A. A. Abdel-Rahman, A. K. Shetty and M. B. Abu-Donia. Pharmacology and Cancer Biology and Neurosciences, Duke University Medical Center and VA Medical Center, Durham, NC.

Histopathological alterations in the brain of adult male rats were evaluated following a daily dermal dose of DEET (40 mg/kg in 70% ethanol) or permethrin (0.13 mg/kg in 70% ethanol) or a combination of the two for 60 days. Control rats received a daily dermal dose of 7% ethanol for 60 days. Animals were perfused and brains were processed for morphological and histopathological analyses following the above regimen. In animals receiving either DEET or Permethrin, degeneration of cerebral neurons was diffusely observed in distinct regions of the motor and somatosensory cortex, the hippocampus, and the cerebellum. In contrast, dying neurons were infrequent in animals receiving both DEET and Permethrin. The density of surviving neurons in cerebral cortex, hippocampus and cerebellar Purkinje cell layer of these animals, however, was dramatically less than control animals, suggesting that in animals receiving both DEET and permethrin neuronal cell death occurred earlier than animals receiving either DEET or permethrin alone. Analysis of glib bellidonic acid protein immunoreactivity revealed significant hypertrophy of astrocytes in all three treated groups with maximal changes in rats receiving both DEET and permethrin. Further, surviving neurons in the latter group exhibited abnormal dendrites. Thus, sub-chronic dermal application of DEET and permethrin either alone or in combination leads to diffuse neuronal cell death in cerebral cortex, hippocampus and cerebellum of the adult brain. The neurotoxic effect is more pronounced when DEET and permethrin are applied together. Collectively, the above alterations can lead to many physiological and pathological alterations, particularly motor and sensory deficits and learning and memory dysfunction. Supported in part by the US Army Medical Research and Materiel Command under Contract DAMD 17-01-1-0470.


The Food Quality Protection Act (FQPA) of 1996 requires the USEPA to consider the cumulative effects of exposure to pesticides having a "common mechanism of toxicity." This presentation reviews published and proprietary data on the neurotoxicity and mechanisms of toxic action of pyrethroid insecticides in mammals as pertains to this statute. The principal effects of pyrethroids in mammals are various signs of excitatory neurotoxicity. Historically, pyrethroids were grouped into two subclasses (Type I and Type II) based on chemical structure and the associated syndrome of intoxication (T (tremor) or C (convulsions) with salivation). However, this classification system is inadequate for identification of common toxic effects. In particular, it does not reflect the diversity of toxic signs evident following oral administration of the various pyrethroids. With respect to mechanisms of toxicity in mammals, pyrethroids act in vivo on a variety of putative biochemical and physiological target sites that merit consideration as sites of toxic action. Voltage-sensitive sodium channels, the sites of insecticidal action, are also important target sites in mammals. However, unlike insects, mammals have many sodium channel isoforms that vary in their biophysical and pharmacological properties, including their sensitivity to various pyrethroids. Pyrethroids also act on some isoforms of voltage-sensitive calcium and chloride channels and these may contribute to toxicity. Effects on peripheral-type benzodiazepine receptors are unlikely to be a principal cause of pyrethroid intoxication but may contribute to convulsions caused by actions at other target sites. Other putative target sites that have been identified in vitro do not appear to play a major role in pyrethroid intoxication. The diverse toxic and pharmacologic effects of pyrethroids suggest that simple additivity models, based on combined actions at a single target, are not appropriate to assess cumulative risk.
1565
AN HPLC METHOD FOR THE DETERMINATION OF MAJOR PERMETHRIN METABOLITES AND PERMETHRIN METABOLISM IN HUMAN LIVER
J. Choi, R. L. Rose and E. Hodgson. Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC. Sponsor: E. Hodgson.
An efficient high performance liquid chromatography (HPLC) method was developed to separate and quantify two permethrin isomers and three major metabolic products (phenoxbenzyl alcohol, phenoxbenzonic acid and phenoxbenzaldehyde), which were produced in studies of in vitro human metabolism using human liver fractions. Two hundred and thirty nanometers was found to be the most sensitive detection wavelength for permethrin isomers and metabolites among 4 previously used wavelengths (210, 230, 254 and 270 nm). Detection limits at 230 nm were 12 nM for phenoxbenzonic acid and 24 nM for all other compounds. A gradient elution using two solvents was used with the initial mobile phase condition of 50% of solvent B (100% water adjusted to pH 1.7 with phosphoric acid) and 50% of solvent A (90% acetonitrile and 10% water, v/v). A pH of less than 2 was required for solvent B to fully protonate and separate an ionizable metabolite, phenoxbenzonic acid. Retention times were 3.7, 4.1, 5.9, 10.2 and 10.5 minutes for phenoxbenzyl alcohol, phenoxbenzonic acid, phenoxbenzaldehyde, trans-permethrin and cis-permethrin, respectively. When trans-permethrin was incubated with human liver fractions, phenoxbenzyl alcohol and phenoxbenzonic acid were the major products. Trans-permethrin incubation with 12 individual human cytochrome P450 isozymes did not produce any significant amounts of metabolites. Addition of NAD+ to human liver microsomal fraction significantly increased the production of phenoxbenzonic acid with a minimum amount of phenoxbenzyl alcohol detected. An incubation of phenoxbenzyl alcohol and phenoxbenzaldehyde metabolism with horse liver alcohol dehydrogenase and yeast alcohol dehydrogenase resulted in significant levels of phenoxbenzaldehyde and phenoxbenzonic acid formation respectively. These results suggested that permethrin hydrolysis products in human liver may be further metabolized by NAD+ requiring alcohol and aldehyde dehydrogenases without cytochrome P450 involvement.

1566
INDUCTION OF CYTOCHROME P450IA1/2 AND P450A4IA ACTIVITIES BY PYRETHROIDS.
M. A. Martinez-Larranga, M. J. Diaz, M. A. Martinez, M. Martinez, M. T. Frejo, V. J. Castellano, R. Fernandez and A. Anadon. Toxicology and Pharmacology, Complutense University, Madrid, Spain.
The objective of the present study was to investigate the effects of repeated exposure to Cyfluthrin or Cyhalothrin, Type II pyrethroid insecticides, on hepatic mixed function oxidase activities in male Wistar rats following oral administration. Animals were treated with Cyfluthrin (20 mg/kg/day orally for 6 days) or Cyhalothrin (8 mg/kg/day orally for 6 days); control animals received orally corn oil. Pyrethroid-treated and control animals were sacrificed 24 hours after the last administration and organs were removed. The livers were individually homogenized and microsomal pellets were prepared and stored at -80°C for enzyme determinations. O-demethylation of 4-methoxresorufin (MROD) and O-deethylony of ethylresorufin (EROD) were determined from appearance of resorufin measured fluorimetrically. Finally, 11- and 12-hydroxylations of laurate were also determined on the microsomal fraction by a HPLC method with radiochemical detection. Effects of pyrethroids on rat hepatic mixed function oxidase system: Results are significantly different from the control value at P<0.05, **P<0.01, or ***P<0.001. These findings may provide a valuable contribution for risk assessment of these insecticides. This work has been supported by projects No. PB97/01236, (DICYCT), No. 08.8/0002/98 (CAM) & No. 99/0936 (FIS), Spain.

1567
COMPARISON OF DIETARY AND ORAL GAVAGE ADMINISTRATION OF BIFENTHRIN IN DEVELOPMENTAL TOXICITY STUDIES IN SPAGUE-DAWLEY RATS.
Bifenthrin, a synthetic pyrethroid insecticide/miticide, was tested in two developmental toxicity studies in Sprague-Dawley rats. Bifenthrin was administered at 5 mg/kg/day via gavage to groups of 25 rats as a solution in corn oil at dosages of 0.5, 1.0 and 2.0 mg/kg/day on days 6 through 15 of gestation. Intermittent tremors were seen among animals receiving 2.0 mg/kg/day between days 10 and 19 of the study. There were no treatment-related effects observed on body weight gains, food consumption, pregnancy, number of implantations, resorptions, litter size, external or internal anomalies (c-sections on day 20). There were no effects at 1.0 mg/kg or below. In the second study, bifenthrin was administered in the diet at dose levels of 0, 30, 90 and 270 ppm on days 6-20 of gestation. Treatment-related clinical signs included tremors, hyperactivity and prolongation of forelimb extension time at 200 ppm. Significant reductions in maternal body weights and gains, adjusted maternal body weights and gains, food consumption were noted at 200 ppm. At c-section on day 20, there were no significant differences in numbers of implantations, pre-implantation loss, litter size, the percentage of live and dead fetuses, percentage of early and late resorptions, fetal body weights and sex ratios or fetal or litter incidence of external, visceral or skeletal findings. The No Observed Effect Level was 90 ppm (7.4 mg/kg/day), based on clinical signs at 200 ppm (16.3 mg/kg/day). The use of corn oil as a vehicle allows for dosing using oral gavage and measurement of the dose each animal receives, however, it results in delivery of a bolus dose into the stomach in an unrealistic vehicle. Administration of bifenthrin in the diet more closely approximates the route of exposure for the human population exposed to small amounts of bifenthrin in their diet.

1568
PESTICIDE TOXICITY IN THYMOCYES IS RELATED TO OXIDATIVE STRESS.
S. Olguin and P. M. Mira. Biomedical Sciences and Pathobiology, VA Tech College of Veterinary Medicine, Blacksburg, VA.
Peptides are widely used in the environment, and exposure to multiple pesticides is very likely, either concurrently or sequentially, over one's lifetime. We have found that selected peptides lindane (1), an organochlorine, malathion (M), an organophosphate, and permethrin (P; a pyrethroid) are cytotoxic to murine (C57BL/6) thymocytes in both concentration- and time-dependent manners, in vitro. Based on equivalent (i.e., 4 µM) concentration (lindane), the following concentrations were chosen: 50 µM L, 75 µM M, 150 µM P, 50 µM L + 75 µM M mixture, and 50 µM L + 150 µM P mixture. These concentrations of peptides caused 16.6, 21.5, 29.0, 59.8 and 53.3% cell death (examined by 7-aminoactinomycin-D assay), respectively. To investigate the role of oxidative stress in causing cytotoxicity, we monitored the pro- and anti-oxidant status of cells. The generation of hydrogen peroxide (H2O2), an indicator of pro-oxidant status of cells, was monitored by CMH, DCFHDA assay. Exposure to above concentrations of pesticides for 15 min augmented the H2O2 production in cells (L 37.2, M 39.2, P 40.5, L+M mix 52.4 and L+P mix 50.6% over control). The effects of above concentrations of pesticide exposure to the cells for 12 hr on activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rx) enzymes were studied to evaluate the antioxidant status of the cells. The activity of CAT was not significantly altered by these treatments. SOD levels were not altered by individual pesticide exposure, but mixtures of pesticides caused increase in SOD levels (Units/mg L 29.1, M 29.4, P 28.0, L+M mix 33.7, L+P mix 31.1). GSH-Px levels in cells with individual pesticides were not altered by individual pesticide exposures, but significantly reduced with pesticides mixtures (GSH-Px Units/mg L 30.5, 28.7, 32.8, 23.2, 21.8; GSH-Rx Units/mg L 33.5, 36.1, 36.6, LM mix 25.7, LP mix 26.1). These results indicate that lindane, malathion and exposure cause immunotoxicity, at least in part, via oxidative stress.

1569
TOXICOLOGICAL ASSESSMENT OF THE OXANILIC ACID METABOLITE OF ALACHLOR.
J. M. Kenneberg, W. F. Heyden and J. L. Honegger. Monsonia Company, St. Louis, MO.
Alachlor is a selective herbicide used on corn and other crops. The 3-aminopyridine acid metabolite of alachlor (alachlor OX) is an environmental degrade that is formed in soil by microbial action and is occasionally detected in ground and surface water. Thus, a number of toxicological studies were conducted with alachlor OX in order to evaluate the potential human or ecological effects that may be associated with this metabolite. These included acute and subchronic studies in rats, a battery of in vitro and in vivo genotoxicity studies, and acute studies in fish, aquatic invertebrates, earthworms and algae. These studies demonstrated that alachlor OX is relatively non-toxic to all species tested. Mortality occurred in rats following a single oral dose of 5000 mg/kg. The No-Observe Effect Levels (NOEL) in 28-day and 90-day rat feeding studies were 10, 000 ppm (approximately 792 mg/kg/day) and 13, 000 ppm (approximately 922 mg/kg/day) (highest level tested), respectively. No evidence of genotoxicity was observed in Ames, mouse lymphoma tests, in vitro chromosomal alteration or mouse micronucleus assays. Alachlor OX was also practically non-toxic in acute studies with several aquatic and terrestrial organisms. No mortality was observed in rainbow trout (Oncorhynclus mykiss), Daphnia magna or earthworms (Eisenia fetida) at the high-
est concentrations tested (100, 95 and 1000 ppm, respectively). The EC_{50} values in Selenastrum capricornutum and Nasella pelliculosa were greater than 100 ppm. Thus, these results demonstrate that the acute and subchronic toxicity of alachlor OXA is substantially less than that of parent alachlor, and indicate that the very low levels of alachlor OXA which are sometimes found in ground and surface water will not pose a significant risk to human health or the environment.

HEPATIC RESPONSES TO CHLOROACETANILIDE HERBICIDE α-GLUCORONIDE DISPLACEMENT DISTINGUISHED BY INDUCTION WITH BOTH ALACHLOR AND METOLACHLOR.

S. R. Miranda1, S. B. Dalton2, R. A. Wormser1 and S. A. Meyer1. 1Toxicology Unit, University of Louisiana at Monroe, Monroe, LA; 2Univ. of Nebraska Medical Center, Omaha, NE and 3National Cancer Inst., New York, NY. 3National Cancer Inst., New York, NY.

Preclinical studies indicate that acute hepatotoxicity of alachlor [2-chloro-2', 6'-di-ethyl-N-mesitylacetanilide]; > 400 mg/kg has been observed by male Long Evans rats as associated with sustained depletion of reduced glutathione (GSH). However, comparable GSH loss occurs with metolachlor [2-chloro-2'-N(4-methylphenyl)-N(2-methyl-4-chlorophenyl)acetanilide; ≥ 2.6 mg/kg] in the absence of acute hepatotoxicity (Dalton et al., Toxicol. Sci. 60:54, 2001). Unlike alachlor, metolachlor is not metabolized to quinonemine, but both chloroacetanilides contain an α-ethyl chloride group. In the present studies, neither alachlor nor metolachlor induced oxidative damage, assessed as thiobarbituric acid-reactive malondialdehyde of liver samples (prepared with antioxidant BHT).

Glutathione S-transferase activity (GST), with chlorodinitrobenzene as substrate, showed statistically significant dose-dependent increases 24 hr after exposure to both alachlor and metolachlor, which immunoblot analysis indicated was not due to an increase in oxidant-induced isoform α or μ. Collectively, these data suggest that alachlor-induced alpha GST results from competition of the α-ethyl chloride group by nucleophilic substitution and is insufficient to cause oxidant-mediated hepatotoxicity. Additional comparative studies using enzymatic and immunoblot analyses of cytochrome P450s indicated that alachlor and metolachlor (0.5 mmol/kg, 4 daily doses) induced CYP2B2/1 to a similar extent, but neither induced CYP1A, 1A2 or 3A1. In conclusion, sustained GSH depletion and induction of GST and CYP2B2/1 by metolachlor, but in the absence of hepatotoxicity, indicate that these responses are related to adaptation to chloroacetanilide exposure. Further, they suggest that alachlor hepatotoxicity can be attributed to a structural group other than the α-ethyl chloride. (Support: NIH ES07046 & University. La Monroe Coll. Pharmacy)

COMPARATIVE IN VITRO AND EX-VIVO STUDIES ON PROPAIN MYELOTOXICITY.

M. Martacchi1, L. Manza2, T. Coccini3, L. Grigolo4 and A. F. Castoldi1. 1Toxicology Division, Magueri Foundation, Pavia, Italy; 2Dept. of Internal Medicine, University of Pavia, Pavia, Italy and 3ECVAM, EC Joint Research Centre, Ispra, Varese, Italy.

3', 4'-Dichloropropionanilide (propanil) has been shown to possess methemoglobinemic activity in both animals and humans possibly through its metabolites 3', 4'-dichloroanilin (DCA) and N-hydroxy-3', 4'-DCA. Propanil also exerts immunotoxic and myelotoxic effects in experimental models. The aim of this study was to compare in vitro and in vivo myelotoxic effects of propanil and its metabolite DCA on mouse bone marrow (BM) precursors by means of colony forming unit (CFU) assays of the myeloid - granulocyte and macrophage - lineages (CFU-GM), the erythroid lineage (CFU-E) and the less mature burst-forming unit erythroid (BFU-E). (1) To compare the in vitro data obtained in ex-vivo experiments performed on BM cells isolated from mice exposed acutely to propanil in vivo. In vitro assays, propanil and DCA concentration-dependently reduced the number of erythroid colonies. Propanil 1CS0s were 62 ± 12 and 18 ± 11 microM in the CFU-E and BFU-E assays, respectively, while those of DCA were 26 ± 7 and 13 ± 5 microM, respectively. Propanil and DCA were equally potent inhibitors of CFU-GM colony formation in vitro (CS0s = 170 microM). In vivo exposure to a single dose of propanil (0, 50, 100 and 200 mg/kg; i.p.) caused a dose-dependent decrease of CFU-E, BFU-E and CFU-GM progenitors 7 days post-exposure. Compared to controls, 100 and 200 mg/kg propanil reduced CFU-E colony formation by approximately 40 and 50%, respectively, and BFU-E by 24 and 39%, respectively. CFU-GM colony formation was reduced to a similar degree. Peripheral blood parameters were not affected by any dose of propanil 1 week after injection. In summary, propanil elicits myelotoxic effects both in vitro and in vivo. The higher potency of DCA toward the BFU-E cells compared to that of propanil suggests a role for propanil metabolites in its toxicity.

CLODINAFOP IS A PEROXISOME PROLIFERATOR IN THE MOUSE LIVER.

E. W. Weber1, B. Lang2, E. Warcher1, L. Steixens3 and C. F. Trendelenburg1.

1Syngenta Crop Protection, Basel, Switzerland and 2Syngenta Crop Protection, Greenboro, NC.

Clodinafop-propargyl (CLF) induced hepatoceleulenic neoplasms at high doses in male mice. In the absence of any mutagenic activity, the induction of liver tumors was considered to be due to a non-genotoxic mechanism. Increased liver weight and hepatocellular hyperplasia were observed in subchronic studies, suggesting an induction of liver enzymes and a mitogenic response. Two studies with male mice were initiated comprising biochemical liver parameters (cytochrome P450 monooxygenases and peroxisomal fatty acid oxidation) and bronchoalveolulinar-based cell proliferation analysis (treatment periods up to 28 days). Major treatment-related biochemical alterations comprised an induction of cytochrome P450 isoenzymes of gene subfamily Cytp as indicated by a strong increase in immunohistochemically detectable Cyp3a proteins and by associated lauric acid 12-hydroxylase activity. Peroxisomal fatty acid oxidation activity was markedly increased. Cell proliferation analysis revealed a strong but transient stimulation of hepatocellular proliferation, accompanied by hepatocellular hyperplasia. In addition, morphometry
of hepatocellular peroxisomes revealed an increase in number and percent area of these organelles. These data identify CLP as a strong peroxisome proliferator with mitogenic and enzyme inducing activity in the mouse liver. Therefore, the cyto- genic response observed with CLP in the mouse liver is inappropriate for extrapolation to humans.

1576 ASSESSMENT OF THE POTENTIAL INTERACTION OF 1C-THIAMETHOXAM WITH HUMAN P-GLYCOPEPTIDE.
J. T. Stevenson*, L. G. Foss and R. C. Peffer†, Syngenta Crop Protection, Inc., Greenboro, NC, †GENEST Corporation, Woburn, MA and ‡Wake Forest University, Winston-Salem, NC.

P-glycoprotein (PGP, encoded by mdrl) is a member of the ABC transporter superfamily and is expressed in endothelial cells of the capillary bed in the brain, intestine, liver, kidney and other tissues. PGP serves as a barrier to keep toxins out of these tissues by actively pumping chemicals that bind to it out of the cell membrane and out of the cytoplasm. Numerous insecticides have been demonstrated to interact with human PGP [Bain et al., 1997, EHP, 105: 812-818]. In order to evaluate the potential of the new insecticide, thiamethoxam to interact with PGP, two experiments were performed in control and human PGP-expressing LLC-PK1 cells derived from the human kidney. The first, a one-generation experiment with [3H]-thiamethoxam, was performed to determine the apparent permeability (Papp) and bidirectional transport of thiamethoxam in the control and polarized cell monolayers. The Papp (7.4 ± 8.5 × 10^(-6) cm/sec for a time course of 15 to 120 minutes) in the control cell (clone A) monolayers is consistent with a membrane-permeable substance. The polarization ratio of Papp (Papp/papp = 0.99) in the human PGP-expressing LLC-PK1 cells (clone 15B-J) was essentially equal to that in the control cells, at each timepoint measured. In contrast, the 15B-J polarization ratio with digoxin (the positive control for PGP transport) was higher than the control cell ratio, demonstrating that digoxin was transported. The mass balance analysis at 120 minutes resulted with a 94% recovery of BSA. The results demonstrated that thiamethoxam was not transported by PGP. The second experiment (PGP-inhibition) determined that the PGP-facilitated transport of digoxin was not inhibited by co-cubated thiamethoxam, as was evident by the consistent digoxin polarization ratio measured over the thiamethoxam concentration range of 0 to 100 µM whereas, ketoconazole inhibited the digoxin transport. The results of the study clearly demonstrated that thiamethoxam is not a substrate or inhibitor of human PGP-mediated efflux.

1577 BLOCK OF COCKROACH NEURON SODIUM CHANNELS BY INDOXACARB INSECTICIDE.
X. Zhao, T. Ikeda, J. Z. Yeh and T. Narahashi. Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL.

Indoxacarb is a newly developed insecticide with high insecticidal activity and low toxicity to nontarget organisms. Our previous study has shown that indoxacarb at 1 - 10 µM is less potent than its metabolite, DCJW, to block voltage-gated sodium channels in mammalian neurons. However, our knowledge of the mechanism of action of indoxacarb on insect sodium channels is very limited. The present study was undertaken to investigate the actions of indoxacarb and DCJW on tetrodotoxin-sensitive (TTX-S) sodium channels in cockroach thoracic ganglion neurons by the whole-cell patch clamp technique. DCJW at 0.1 - 1.0 µM slowly and irreversibly blocked TTX-S sodium channels. At -100 mV membrane holding potential, 100 nM DCJW reduced the peak amplitude of sodium current by 75.7 ± 12.1% (n = 6). Indoxacarb was less potent than DCJW. No changes in sodium channel activation kinetics was observed after application of indoxacarb or DCJW. Sodium channels of cockroach neurons undergo two kinetically distinct types of inactivation, fast inactivation and slow inactivation. DCJW at 100 nM shifted the half-maximum fast inactivation potential (V1/2) to -72.1 ± 1.6 mV (n = 6) from the control value of -64.0 ± 0.6 mV (p < 0.001) and shifted the V1/2 for slow inactivation to -79.1 ± 3.4 mV from the control of -87.5 ± 3.2 mV (n = 5, p < 0.001). The voltage-gated potassium channel was not affected by indoxacarb or DCJW. These results suggest that the insect sodium channels are more sensitive to indoxacarb and DCJW than the mammalian sodium channels providing the basis for high insecticidal activity and low mammalian toxicity. Supported by NIH grant NS41443.

1578 INSECTICIDAL ACTIVITY OF ESSENTIAL OILS: OCTOPAMINERGIC SITES OF ACTION.
E. E. Enna. Biochemistry, School of Medicine, Vanderbilt University, Nashville, TN.

A study was conducted to determine the insecticidal activity and mechanism of action of three essential oils (eugenol, a-terpineol and cinnamal alcohol) and an equal part mixture (3-blend) against American cockroaches (Periplaneta americana). To address species differences in response to treatment with the test oils, Carpenter ants (Camponotus pennsylvanicus De Geer) and German cockroaches (Blattella germanica) were included in this study. Exposed American cockroaches demonstrated hyperactivity followed by hyperextension of the legs and abdomen, then fast knockdown or quick immobilization followed by death. Ants and German cockroaches showed fast immobilization/knockdown followed by mortality. The 1:1:1 mixture (3-blend) was substantially effective against all test insects. One of the most remarkable observations was the increased frequency of heartbeat of American cockroaches in response to topical application of test oils. The changes in the pattern of cAMP level was biphasic. A significant increase in the cAMP level was found in response to 1 nmole/ml of eugenol, or 3-blend and 10 nmole/ml of a-terpineol. At higher concentrations a significant decrease in cAMP level was found. Blockade of octopamine receptors binding sites was also illustrated at lower concentrations of the test chemicals as judged by the decreased binding activity for 3H-octopamine to its receptors. In conclusion: 1- test oils are neuro-insecticides and their insecticidal activity is species-dependent, 2- a synergistic effect of the three oils was found when they were equally mixed (3-blend), and 3- octopaminergic system mediates the insecticidal activity of eugenol, a-terpineol and the 3-blend.

1579 DOSE CONVERSIONS BETWEEN UNITS OF MG/KG AND MG/M2 FOR CYTOTOXIC ANTICANCER AGENTS: A CASE STUDY REGARDING THE IMPORTANCE OF CONSISTENT ESTIMATIONS OF BODY SURFACE AREA.

The toxicity of cytotoxic anticancer drugs correlates more closely with body surface area (BSA) than with body weight (BW). Thus, it is standard practice to administer and compare such drugs on the basis of BSA. To interconvert between units of mg/kg and mg/m², it is necessary to know either BSA or BW. Accurate values for BW are easy to obtain. In contrast, BSA is difficult to measure, and, therefore, is usually calculated from a known BW. The relationship between BW and BSA is described by the formula BSA(m²) = k * BW(kg)^3/2, where k is a species-specific constant derived from empirical data. In practice, the values for k vary between laboratories (e.g., values for b for the mouse have been found to vary from 0.050 to 0.096). Confusion and miscommunication can occur as a result of the use of different species-specific constants. For example, difficulties have arisen during the dose selection process for a novel cytotoxic anticancer agent with a steep dose-response curve. In this case, if different investigators use b values as varied as 0.099, 0.101, and 0.111 then animal BSA values for a 10 kg dog will vary by up to 12% (0.4631, 0.4724, and 0.5192 m², respectively). Also, although quantities of the anticancer drug are calculated from a target dose set in units of mg/m², the amounts of the drug given to the 10 kg dog will likewise vary, depending on the choice of k (0.926, 0.943, and 1.056 mg/m², respectively, for a 2 mg/m² dose). The use of a single set of species-specific constants by all investigators is proposed, as it would be beneficial in removing confusion from discussions of study designs and results. Such a proposal has an even more immediate benefit in the case of compounds with very steep dose-response curves, where even small variations in dosing can profoundly influence the results obtained and their interpretation.

1580 FOUR WEEK ORAL TOXICITY STUDY OF THE CANCER CHEMOPREVENTIVE AGENT RESVERATROL IN RATS.
P. L. Kurosky, R. L. Morrissey, VI. Hobbart, G. Stern, T. Kong, J. A. Casswell and B. S. Levine, *Toxicology Research Laboratory, University of IL at Chicago, Chicago, IL, †Pathology Associates, Chicago, IL, ‡Rutgers University, Piscataway, NJ and †NCI, Rockville, MD.

Resveratrol, a naturally occurring compound derived from grapes, inhibits cellular events associated with tumor initiation, promotion, and progression, and is being developed as a cancer chemopreventive agent. The present study examined the toxicity of resveratrol following 28 days of daily gavage administration to four groups of 20 rats/sex. Dose levels were 300, 1000, and 3000 mg/kg/day, based on a two-week pilot study. Oral administration of 3000 mg/kg/day resulted in nephrotoxicity observed as elevated serum BUN and creatinine levels, increased kidney weights, and gross and microscopic renal lesions. Dehydration at this dose was supported by reduced body weight gains (secondary to decreased food intake) and hyperthermogenesis. Additional clinical signs in high dose animals included labored breathing, hunched posture, decreased activity, rough coat, diarrhea, soft stool, and red material around the nose. Leukocytosis occurred in males, whereas anemia in females and possibly in males in the 3000 mg/kg/day dose group may have been a direct effect on RBCs (Inc. total bilirubin), or secondary to renal injury (dec. erythropoietin synthesis). Increased serum ALT, ALKP and possibly total bilirubin indicated mild liver toxicity in the high dose group, but these changes were not supported histologically. As
1581 PHARMACOKINETICS AND TISSUE DISTRIBUTION OF THE PROSTATE CANCER CHEMOPREVENTIVE AGENT Lycopene in Male Beagle Dogs.

B.S. Levine1, I.J. Korytko1, M. Szwecie-Sapunziak1, K. A. Redvolld1, P.E. Bowen1, and L. A. Crowell1. 1Toxicology Research Laboratory, Univ. of IL at Chicago, Chicago, IL. 2Human Nutrition, Univ. of IL at Chicago, Chicago, IL.

Lycopene, the predominant carotenoid in tomatoes and tomato products, has been associated with a decrease in prostate cancer risk. The present study was conducted to determine the pharmacokinetics and tissue distribution of lycopene in male dogs following 28 days of oral administration. Prior studies examined the pharmacokinetics of lycopene following single doses of 10, 30 and 50 mg/kg, and 30 and 50 mg/kg/day for 4 consecutive days. The results indicated that the elimination half-life after a single dose of 10 to 50 mg/kg was about 36 hours, whereas the elimination half-life was somewhat shorter after four daily doses. In addition, peak plasma lycopene levels were similar following either 30 or 50 mg/kg/day for four days. Based on these results, 30 mg/kg/day was selected for the main study. An oral dose of 30 mg/kg/day administered to six dogs for 28 days resulted in steady-state plasma concentrations between 400 and 700 ng/mL 96 hours after the first dose. Mean apparent clearance, volume of distribution, and elimination half-life were 2.29 L/hr/kg, 96 L/kg, and 30.5 hours, respectively. Three dogs each were sacrificed one or five days after the last dose and were necropsied for lycopene analysis. Lycopene concentrations were highest in liver, adrenal, spleen, lymph node, and G1 tract (in that order). Mean liver lycopene levels were 35 μg/g one day and 49 μg/g five days after cessation of treatment. Prostate lycopene levels were fairly low, with mean values less than 0.13 μg/g five days after dosing ceased (< 0.4% of liver levels). The long plasma elimination half-life is consistent with greater lycopene levels in tissues five days compared to one day following cessation of treatment. (Supported by NCI Contract No. N01-CN-95055).

1582 DIETARY FOLATE DEPLETION IN BEAGLE DOGS; IS THYMIDYLATE SYNTHASE INHIBITOR TOXICITY INCREASED?

D. B. Colangelo1, C. Runux1, D. A. Williams1, D. J. Meyer1, R. Bendele1, and F. C. Richardson1. 1Life Sciences, Gilead Sciences, Inc., Boulder, CO and 2Texas Agriculture Experiment Station, Texas A & M University, College Station, TX.

The objective of the study was to determine if dietary folate acid depletion in beagle dogs altered the toxicity of the thymidylate synthase inhibitor (TSI), GS7904L (GW18431/29), currently under clinical development as an anticancer drug. Classically, human patients are deficient in nucleotide metabolism, including thymidylate and folate. An association has been made between patient's homeostatic status (a reflection of intracellular folate acid) and enhancement of TSI toxicity. In previous studies with GS7904L, severe toxicity was observed in dogs treated with 2.5 mg/kg (40-60 mg/kg/m2) as a single intravenous administration. To assess if GS7904L toxicity may be exacerbated in association with folate acid deficiency, a model was established to mimic this condition in beagle dogs. Two groups of young, female beagle dogs were fed either a standard certified diet or a diet deficient in folate acid (Teklad TD069) for 21 or 72 days. Both diets were equally palatable. Samples were collected over the course of the study for serum folate, plasma homocysteine and whole blood hematocyte analyzers. Clinical observations and body weights were recorded daily. All dogs were administered 1 mg/kg (20 mg/m2) GS7904L by bolus IV administration on either day 14 or day 66 of the study. At the time of drug administration, the serum folate levels were reduced 25% or 50% from baseline concentrations. Mild hematologic changes to white blood cell counts were noted in both the dogs fed the folate deficient and normal diets and no clinical toxicity was seen in any dog. These findings show that, in the dog under the conditions tested, dietary folate acid depletion did not increase GS7904L toxicity.

1583 EVALUATION OF THE ACUTE TOXICITY OF LIPOSOME-ENCAPSULATED VINCRISTINE SULFATE.

P. Tang1, R. Wehage2, C. Flores3, V. Lee-Brotherton2, M. Lueks2, V. Tal2, and J. Daniel2. 1InnoPharmaceuticals Corporation, Vancouver, BC, Canada, 2Cantox Health Sciences International, Missisauga, ON, Canada, and 3Cantox Health Sciences International, Vancouver, BC, Canada.

Vincristine Sulfate Liposome Injection (VLSI) utilizes sphingomyelin/cholesterol liposomes (55:45) as a systemic drug delivery vehicle for the treatment of neoplastic disease. In this presentation, the acute toxicity of VLSI was evaluated in Sprague-Dawley rats and beagle dogs. Rats (5/sex/dose) were administered single iv doses of 1.1 to 4.7 mg/m2; dogs (1/sex/dose) were administered doses of 0.6 to 2.0 mg/m2. Free VCR, empty liposomes, and saline groups were included in each study. In rats, VLSI was associated with dose-dependent body weight (BW) loss, myelosuppression, and testicular atrophy. There was no mortality among rats. Dogs treated with VLSI also showed dose-dependent BW loss and myelosuppression. Gastrointestinal toxicity was observed in both dogs administered VCR (1.1 mg/m2; graded minimal in male, slight in female), in the male given VLSI at 1.1 mg/m2 (minimal), and in animals given 0.5 mg/m2 VLSI (minimal to slight). The only mortality was the male dog aged 1 year (BW = 2.6 g/m2) at 0.6 mg/m2. At 1.1 mg/m2, BW loss in the free VCR dogs was 7% (male) and 22% (female) greater than in the VLSI-treated animals. The effects of equivalent doses of free VCR and VLSI on myelosuppression were comparable in rats (doses of 2.9 mg/m2) and in dogs (doses of 1.1 mg/m2) in terms of time of onset, severity, and duration. Free VCR and VLSI (1.1 mg/m2) induced similar increases in aspartate aminotransferase levels in dogs, suggestive of muscle injury in the absence of increases in other aminotransferase levels. These data suggest that dogs may be more sensitive than rats to the toxic effects of VCR, whether free or liposomal, and that there are no new, unexpected acute toxicities observed for VLSI when compared to free VCR.

1584 A SINGLE DOSE INTRAVENOUS TOXICITY STUDY OF A LYMPTOXIN BETA RECEPTORAGONISIANTIBODY IN THE CYNOIMOLUS MONKEY.

M. T. Cooper1, L. Bernier2; and L. D. Green3. 1Preclinical Development, Biogen, Cambridge, MA and 2Covance Laboratories, Vienna, VA.

LTBR mAb, a murine antibody with agonist activity toward the lymphotoxin beta receptor (LTβR), is being developed as a therapeutic for LTβR expressing solid tumors. Administration of the LTBR leads to cytokine release and death of tumors. A single intravenous injection of LTBR mAb in male cynomolgus monkeys at a dose level of 0.2 or 2.0 mg/kg had no effect on the study parameters. There were no abnormal clinical observations noted immediately following dose administration or over the subsequent 7-21 day observation period. Body weight and body temperatures recorded were within normal variation for cynomolgus monkeys. LTBR mAb had no effect on the delayed hypersensitivity (DTH) response or keyhole limpet hemocyanin (KLH) evaluations, as compared variations were seen in all groups. The hematologic, coagulation results, differential leukocyte counts, cellular morphology, and clinical chemistry parameters were unremarkable and similar among all groups on days 8 and 22. Serum concentrations of LTBR mAb at 2 hours post-dose were below the limit of quantitation in animals treated with 0.2 mg/kg LTBR mAb and were 41-52 μg/mL in animals treated with 2.0 mg/kg LTBR mAb. Antibodies to LTBR mAb were detected by day 8 in all LTBR mAb-treated animals. Findings noted at necropsy or identified during histopathologic examination were those known to occur as spontaneous changes in cynomolgus monkeys or associated with administration of incomplete Freund’s Adjuvant (used for DTH immunization). LTBR mAb administration did not affect the incidence or character of these findings. Based on the results of this study, a single intravenous administration of 2.0 mg/kg LTBR mAb was well tolerated.

1585 SAFETY OF A RIBOZYME TARGETING HER2 RECEPTOR mRNA (HERZYM 9™) IN CD-1 MICE AND CYNOIMOLUS MONKEYS FOLLOWING DAILY BOLUS SUBCUTANEOUS INJECTIONS FOR 29 DAYS.


A ribozyme (HERZYM 9™) targeting the mRNA of the human epidermal growth factor receptor-2 (HER2) has been developed. Two toxicity studies were performed in CD-1 mice and cynomolgus monkeys. Animals received daily subcutaneous (SC) injections for 29 days of HERZYM 30 (30, 100 or 300 mg/m2/day). In CD-1 mice, HERZYM was well tolerated with no effects on clinical chemistry, hematology, viability, clinical observations, body weights, food consumption or gross pathology. HERZYM-related increases in liver weights were seen in mid- and high-dose females that resolved in the recovery groups. Cytoplasmic basophilia of renal cortical tubules and Bowman’s capsule epithelial cells in the kidneys was observed for all HERZYM-treated groups and resolved in all but the high-dose group at recovery. In monkeys, there were no effects of HERZYM treatment on body weight, urinalysis, heart rate, blood pressure, ECG, clinical chemistry, clinical hematology or coagulation parameters. HERZYM-related changes in organ weights were: decreased thymus weights and slightly increased kidney weights in all treatment groups. The former was associated with microscopic findings of thymic atrophy and resolved completely in recovery animals. Accumulation of basophilic
granules in renal tubular epithelium and minimal to mild renal tubular degeneration was seen in most HERZYMETH287-RECEPTOR ANTAGONIST 1 IN MALE AND FEMALE RHEUS MONKEYS WITH A 4-WEEK RECOVERY PERIOD.

C. P. M. Hopper, J. Newgren, E. Cheung, E. Bumstein-Benton, M. Bore, L. Neylon, S. Rousselle and T. Reynolds, Preclinical Development Department, Bayer Corporation, Center for Biotechnology Research, Berkeley, CA. "MRI Biomedical, A Division of Charles River Laboratories, Inc. Sparks, NV and Pathology Associate, Charles River Company, Frederick, MD.

BAY 16-9996 is a recombinant human interleukin-4 receptor antagonist being developed for the treatment of severe asthma. It is designed to compete with endogenous interleukin-4 for the binding of the IL-4 receptor, resulting in a switch from a TH2 cytokine secretion profile to a TH1 cytokine profile. To support ongoing clinical development, BAY 16-9996 was administered to male and female rhesus monkeys by subcutaneous (SC) injection every other day for 13 weeks followed by a 4-week recovery period at doses of 0.3, 1.0 and 3.0 mg/kg (4–6×/group). BAY 16-9996 produced no apparent toxicity. There were no changes in clinical observations, food consumption, body weights, serum chemistry, hematology, coagulation, ophthalmology, organ weights, gross pathology, and microscopic pathology. BAY 16-9996-related findings were limited to injection site reactions: minimal to moderate perivascular infiltrates of mononuclear cells, and hemorrhage and fibrosis, which were largely reversible at the end of the recovery period. A dose-dependent increase in the incidence of BAY 16-9996-specific antibodies was observed at three weeks post initiation of treatment. The magnitude of antibody response correlated with an increase in circulating levels of BAY 16-9996. In summary, BAY 16-9996 was well tolerated in male and female rhesus monkeys with no signs of systemic toxicity, which was administered by SC injection every other day, at doses of up to 3.0 mg/kg for 13 weeks.

3-MONTH INHALATION TOXICITY STUDY WITH sIL-4R IN CYCLOMOLUS MONKEYS.


Immune function and general health were evaluated in cynomolgus monkeys administered human sIL-4R receptor via inhalation exposure (closed face mask system) or intravenous (IV) injection twice weekly for 12 weeks with a 12-week recovery period. Studies animals were divided into four groups: placebo inhalation, low dose inhalation (0.1 mg/kg), high dose inhalation (1 mg/kg), or IV (0.2 mg/kg). Animals were evaluated for effects on clinical signs, body weights, ophtalmology, hematology, clinical chemistry, urinalysis, pharmacokinetics and antibody response to the drug. Cellular immune function was assessed using delayed-type hypersensitivity (DTH) testing. Humoral immune function was assessed by measuring antibody response to keyhole limpet haemocyanin (KLH). Lymphocyte subpopulation analysis (CD3, CD4, CD8, CD14, CD20, CD56, and CD69) was conducted using flow cytometry of whole blood. All animals were subjected to a detailed necropsy, histological and immunohistochemical evaluations. There were no clinical signs, ophthalmic findings or differences in body weight that could be attributed to administration of sIL-4R. Clinical pathology evaluation revealed no discernible treatment-related effects. Immune function was not affected by treatment with sIL-4R as assessed by DTH, antibody response to KLH, or by lymphocyte subset analysis. Antibody response was seen in 90% of animals receiving drug by inhalation; 20% of animals receiving drug IV. Exposure levels decreased with time in the inhalation-treated groups, most likely due to antibody development. Exposure levels were maintained in the IV treated group. Terminal investigations did not reveal an effect of sIL-4R administration. Immunohistochemistry evaluations of lymphoid tissues revealed no differences in T and B lymphocyte populations. In conclusion, there was no evidence of systemic effects or evidence of alteration in immune function in animals administered human sIL-4R either by inhalation or IV for 12 weeks.

DIFFERENTIAL SENSITIVITY OF 4 SPECIES TO OBSTRUCTIVE NEPHROPATHY PRODUCED BY AMDOXOVIR, A NUCLEOSIDE ANALOG WITH POTENT ANTIVIRAL ACTIVITY AGAINST HIV.


1-B-D-2, 6-diamino purine dioxolane (Amdoxovir, DAPD) is in Phase I/II clinical trials for treatment of HIV. Amdoxovir is rapidly absorbed after oral dosing and deaminated by adenosine deaminase to yield dioxolane guanosine (DXG), the active anti-HIV agent. One-month toxicity studies in mice and marmosets supported an IND. Amdoxovir produced obstructive nephropathy at doses ≥ 90 mg/kg in mice and 120 mg/kg in marmosets. One-month studies were also con-
1591 PHARMACOKINETIC DISPOSITION IN ARTHROPATHY OF JUVENILE DOGS INDUCED BY A QUINOLONE ANTIMICROBIAL AGENT.

K. Yabe\(^{1,2}\), K. Yoshida\(^{1}\), S. Nishida\(^{1}\), M. Sekiguchi\(^{1}\), T. Sugawara\(^{1}\), K. Furutani\(^{1}\), M. Goery\(^{1}\) and K. Okada\(^{3}\), Yoko Reiko Center, Daiichi Pharmaceutical Co., Ltd., Edogawa-ku, Japan and \(^{1}\)Department of Veterinary Pathology, Iwate University, Morioka, Japan.

Quinolone antimicrobial agents including oxofloxacin induce arthropathy in juvenile animals, but not in adult animals. To examine the age-specific difference in susceptibility to oxofloxacin arthropathy, its pharmacokinetic disposition and early pathological change in the articular cartilage were compared between male juvenile (3-month-old) and adult (18-month-old) dogs. Oxofloxacin was orally administered at 5, 10 or 20 mg/kg/day to these dogs for 8 consecutive days, and serum and cartilage drug concentrations were periodically analyzed by HPLC. Serum Cmax and AUC0-24h, and drug concentrations in the cartilage of juvenile dogs were increased in a dose dependent manner. At 20 mg/kg/day, the drug disposition of juvenile dogs was equal or lower than that of adult dogs. Macroscopically, arthropathy characterized by fluid-filled vesicles in the articular surface was observed only in the juvenile dogs receiving 10 and 20 mg/kg/day, but not 5 mg/kg/day. Microscopically, the juvenile dogs showed cavity formation with unmasked collagen fibers in the middle zone of the articular cartilage and numerous degenerative or necrotic chondrocytes around the cavity on day 2, followed by appearance of chondrocytes clusters on day 8. Meanwhile, the adult dogs represent the thin and zonal different cartilage layer, in which the typical tidemark was observed, suggesting that the existence of the mature articular cartilage tissue may related to protective action on arthropathy. Based on these results, high susceptibility to arthropathy in juvenile dogs can not be explained only by drug disposition to the articular cartilage. The interaction between oxofloxacin and immature cartilages on cell cycles and matrix synthesis may be important for pathogenesis of arthropathy.

1592 REPEAT-DOSE TOXICITY STUDIES IN RATS WITH TWO SUSTAINED RELEASE FORMULATIONS OF LY307161, A PROTEASE-RESISTANT GLP-1 ANALOG.

R. A. Byrd and K. F. Mace, Toxicology and Drug Disposition, Eli Lilly and Company, Greenfield, IN; Sponsor: T. Sauer.

LY307161 is a protease-resistant analog of Glucagon-Like Peptide 1 (GLP-1) that is under development for treatment of Type 2 diabetes. Two sustained release formulations of LY307161, designated NDP-1 or NDP-2, were evaluated in separate 1-month rat studies. F344 rats (8-12/sex/group) were given daily subcutaneous doses of 0, 1, 4, or 20 mg/kg. GLP-1 pharmacodynamic effects observed with NDP-1 included decreases in body weight (45-16%), food consumption (46-16%), and triglycerides (19-46%) at all doses. No target organ toxicity was identified with NDP-1. Toxicologically important findings with NDP-1 were limited to injection site reactions in the 4- and 20-mg/kg groups characterized by swelling, minimal-to-marked granulomatous inflammation, and correlated changes in clinical pathology consistent with the inflammation. Injection site reactions in the 1-mg/kg group were of lesser incidence and magnitude, were not associated with clinical pathology changes, and were not clinically apparent. On Day 29, AUC0-24h values for the 1-, 4-, and 20-mg/kg groups were 232, 843, and 2649 ng*h/mL, respectively. With NDP-2, GLP-1 pharmacodynamic effects also included decreases in body weight (47-14%), food consumption (47-18%), and triglycerides (47-39%). No target organ toxicity was identified with NDP-2. Injection site reactions with NDP-2 occurred only in the 4- and 20-mg/kg groups without clinical pathology changes, and the severity of the reactions, characterized as minimal-to-moderate, was reduced relative to NDP-1. Plasma exposure with NDP-2 was higher relative to NDP-1; the AUC0-24h values on Day 33 for the 1-, 4-, and 20 mg/kg groups were 380, 1247, and 4424 ng*h/mL, respectively. In conclusion, NDP-2 exhibited improved tolerability and bioavailability relative to NDP-1 in rats. The NOEL for target organ toxicity with either LY307161 formulation was 20 mg/kg. Plasma exposure (AUC) at the NOEL was 284- to 49-fold that of maximum anticipated human exposure thus demonstrating an adequate Margin of Safety.

1593 REPEAT-DOSE TOXICITY STUDIES IN DOGS WITH TWO SUSTAINED RELEASE FORMULATIONS OF LY307161, A PROTEASE-RESISTANT GLP-1 ANALOG.


LY307161 is a protease-resistant analog of Glucagon-Like Peptide 1 (GLP-1) that is under development for treatment of Type 2 diabetes. Two sustained release formulations of LY307161, designated NDP-1 or NDP-2, were evaluated in separate 1-month dog studies. Beagle dogs (3/sex/group) were given daily subcutaneous doses of 0, 0.2, 0.8, or 3 mg/kg. GLP-1 pharmacodynamic effects observed with NDP-1 included vomiting, decreased body weight (41-79%), and decreased qualitative food consumption at all doses. No target organ toxicity was identified with NDP-1. Toxicologically important findings in the 0.8- and 3-mg/kg groups were limited to injection site reactions characterized by swelling, moderate-to-marked pyogranulomatous inflammation, and correlated changes in clinical pathology consistent with the inflammation. Injection site reactions in the 0.2-mg/kg group were of lesser magnitude, were not associated with important clinical pathology changes, and were not clinically apparent. On Day 29, AUC0-24h values for the 0.2-, 0.8-, and 3-mg/kg groups were 71, 210, and 620 ng*h/mL. With NDP-2, GLP-1 pharmacodynamic effects also included vomiting, decreased body weight (43-9%), and food consumption (41-23%) at all doses; no target organ toxicity was identified with NDP-2. Toxicologically important findings were again limited to injection site reactions at all NDP-2 doses. Sur the severity of the reactions, characterized as minimal-to-moderate pyogranulomatous inflammation, was reduced relative to the NDP-1 formulation. Correlative clinical pathology changes were also diminished relative to NDP-1. On Day 28, AUC0-24h values for the 0.2-, 0.8-, and 3-mg/kg groups were 52, 137, and 349 ng*h/mL. In conclusion, LY307161-2 exhibited an improved local tolerability profile in dogs. The NOEL for target organ toxicity with either LY307161 formulation was 3 mg/kg. Plasma exposure (AUC) at the NOEL was 35- to 63-fold the maximum anticipated human exposure thus demonstrating an adequate Margin of Safety.

1594 HEPATIC PEROXISOMAL PROLIFERATION AND MICROSIONAL ENZYME INDUCTION BY THE LIPID-REGULATING AGENT CI-1027 IN RODENT AND NONRODENT.

R. M. Walker\(^{1}\), L. M. King\(^{1}\), F. J. McGuire\(^{2}\), D. Mant\(^{1}\) and L. Radulovic\(^{1}\), Drug Safety Evaluation, Pfizer Global Research & Development, M Amouss, ON, Canada and Drug Safety Evaluation, Pfizer Global Research & Development, Ann Arbor, MI.

CI-1027 [6, 6'-oxybis[2, 2-dimethyl-4-hexanoyl]acid] is a dicarboxylic acid lipid-regulating agent in experimental models of dyslipidemia. Hepatic peroxisome proliferation and microsomal enzyme induction were evaluated after oral CI-1027 administration for 2 weeks in beagle dogs, 4 weeks in Wistar rats and cynomolgus monkeys, and 13 weeks in B6C3F1 mice. Peroxisomal enzyme activity markers were carnitine acetyltransferase, acyl CoA oxidase, β-oxidation, and catalase, and microsomal enzyme activity markers included laric acid hydroxylase, an activity marker for CYP7A1, and UDP glucuronate transferase (GT). No hepatic changes occurred in dogs given doses up to 1000 mg/kg. In monkeys, liver weight increased at 300 mg/kg, but was unchanged at 10 to 100 mg/kg. However, there was no pattern of enzyme changes indicative of peroxisomal proliferation or microsomal enzyme induction in monkeys. In rats at all doses, 30 to 750 mg/kg, liver weight increased up to ~100% and peroxisomal enzyme activities up to 6-fold. Laric acid hydroxylase increased up to 11-fold at all doses in rats and GT activity increased up to 1.5-fold at 100 to 750 mg/kg. Increased CYP7A1 concentrations were confirmed with an ELISA. In mice, liver weight increased up to 25%, peroxisomal enzyme activities increased 48% to 4.7-fold, and microsomal laric acid hydroxylase increased 3- to 10-fold at all doses [500, 1000, and 2000 mg/kg]. Peroxisome proliferation was confirmed by ultrastructural examination. Toxicokinetic profiles revealed that CI-1027 exposures were less than dose-proportional in all species. It is concluded that CI-1027 is similar in related compounds that induce hepatic peroxisome proliferation and microsomal enzyme induction in rats and mice, but not in nonrodent species.
1595 PRECLINICAL SAFETY EVALUATION OF THE LIPID-REGULATING CANDIDATE CI-1027 IN CYTOMEGALIC MONKEYS AFTER 4 AND 26 WEEKS OF ORAL DOSING.


The sub-acute and chronic effects of CI-1027 (6, 6-oxysterol[2, 2-dimethyl-4-oxaneoic acid]-Ca), a peroxisome proliferator in rodents, was investigated in male and female cytomegalic monkeys receiving 10, 30, 100, or 300 mg/kg daily by gavage for 4 weeks, or 10, 30, or 100 mg/kg for 26 weeks. In the 4-week study, 1 male and 2 females at 300 mg/kg were euthanized mid-bond during Week 3 or 4. Clinical laboratory changes consisted of decreased RBC count/hematocrit/hemoglobin in females at 100 and 300 mg/kg (10% to 27% from pretest), and increased APTT (41% to 72%) and PT (14%) in males at 100 and 300 mg/kg. Pathological changes included increased relative liver weight at 300 mg/kg (58% to 93%), hepatic cellular hypertrophy and/or hepatocellular fatty change, and decreased fine vacuolation of the adrenocortical zona fasciculata at 100 and 500 mg/kg. There was no hepatic microsomal or peroxisomal enzyme induction, indicating absence of peroxisome proliferation. In the 26-week study, RBC count/hematocrit/hemoglobin were 11% to 18% lower in males and females at 100 mg/kg. Slight decreases in mean corpuscular volume (7.1%) and mean corpuscular hemoglobin (9.2%) were also noted in males at 100 mg/kg. Increase in absolute (32% to 34%) and relative (46% to 45%) liver weight and relative kidney weight (33% to 43% of controls) were noted in males and females receiving 100 mg/kg. Microscopic findings consisted of a dose-related increase in the relative incidence and severity of lipid vacuolation in the liver of all males and females receiving 30 and 100 mg/kg. Lipid vacuolation was also noted in the kidneys of 2 animals at 30 mg/kg, and all animals at 100 mg/kg. CI-1027 appears to act similar to other peroxisome proliferators in primates.

1596 TOXICOKinetic-TOXICodynamic RELATIONSHIPS IN HUMAN PHENOBarbital POISONINGS.

B. Mégare, B. E. Baud, S. W. Burton, and C. Bismuth. "Intensive Care Unit, Lariboisiere Hospital, Paris, France."

The value of PK-PD relationships in clinical pharmacology is now well recognized. However, the potential interest of Toxicokinetic-Toxicodynamic (TK-TD) relationships in medical toxicology has been poorly investigated. The aim of this study was to correlate the depth of coma with plasma phenobarbital (PB) concentrations. Materials and Methods: Plasma PB concentrations were measured using an enzymatic assay. The depth of coma was assessed using the Glasgow coma scale (GCS). Non-linear regression was used for modeling TK-TD relationships. Results: TK-TD relationships were studied in 6 acute PB poisonings. Two patients were previously treated with PB. Previous treatment was unknown in 2 patients. Mixed drug poisoning was noted in 2 patients. The GCS at the time of hospital admission was 5 in the 6 patients, the mean plasma PB concentration was 710.5 ± 281.9 μg/ml (range: 366 - 1030). The TK-TD relationship was well fitted with the sigmoidal Enzyme model. In the 6 patients, the mean Hill coefficient (mH) was 0.6 ± 1.3 and the mean C50 was 289.7 ± 97.1 μg/ml (range: 170 - 440). Discussion: A maximal toxic effect (GCS of 3) was associated with a wide range of plasma PB concentrations. During the course of PB poisoning, the relationship between the depth of coma and the corresponding plasma PB concentration is of a sigmoidal shape. The Hill value of the Hill coefficient (mH) showed that a small decrease in plasma PB concentrations near the C50 was associated with a dramatic improvement in their level of consciousness. In the 6 patients, the mean C50 was approximately equal to 3-fold the upper limit of the therapeutic plasma concentration of PB given by our toxicological laboratory (< 100 μg/ml).

1597 CLINICAL SYNDROME ASSOCIATED WITH ALPRAZOLAM INGESTION IN DOGS: 238 REPORTS (JANUARY 1998-AUGUST 2001).

T. A. Wetter, J. A. Richardson and L. C. Albrecht. ASPCA Animal Poison Control Center, Urbana, IL.

Alprazolam is a benzodiazepine that is used as an anti-anxiety agent. Alprazolam acts at the limbic, thalamic, and hypothalamic levels of the CNS and has anxiolytic, sedative, hypnotic, skeletal muscle relaxant, and anticonvulsant properties. A retrospective study was conducted of alprazolam ingestion in dogs that were reported to the ASPCA Animal Poison Control Center between January 1998 to August 2000. Data analysis included amount ingested, clinical effects, and time of onset of signs. Two hundred and thirty-eight cases of suspected alprazolam toxicoses in dogs (ranging in age from 11 weeks to 13.5 years) were evaluated. Approximate ingested sedative dosages ranged from 0.01 to 5.55 mg/kg. Of the two hundred and thirty-eight cases of suspected toxicity, there were 108 males (45%) and 113 females (47%). Gender information was not available in 17 dogs (7%). Incidence of clinical signs reported included: ataxia/diarrhoea (58%, 161 cases), depression (34%, 83 cases), hyperactivity (33%, 30 cases), vomiting (85%, 19 cases), weakness (78%, 15 cases), tremors (5%, 13 cases), and vocalization (4%, 10 cases). Other signs reported included tachycardia, tachypnoea, hyperthermia, diuresis, and increased salivation. In 38% of cases, clinical signs developed within 10 - 30 minutes. Treatment of overdoses would include standard decontamination procedures, such as emetics and activated charcoal. Flumazenil is a specific benzodiazepine antagonist and could be used to treat dogs with severe CNS depression.

1598 ATTENUATION OF HEPATIC FIBROSIS OBSERVED WITH A "GUTLESS" ADENOVIRAL VECTOR.

L. M. King1, J. B. Markovits1, and R. M. Lyons2. 1"Genetix Therapies, Inc., Gaithersburg, MD and 2"Novartis Pharmaceuticals, East Hanover, NJ.

Adenoviral vectors are being explored as potential therapeutic agents, although clinical utility has been limited due to the adverse effects seen in liver following intrahepatic injection. In previous studies, we demonstrated in male monkeys and CD-1 mice that an early generation adenoviral vector caused hepatocellular apoptosis and activation of hepatic stellate cells with subsequent fibrosis. In this study, in order to determine if viral gene expression contributes to hepatic toxicity, male and female CD-1 mice were injected with 6 x 10^7 (low dose), 3 x 10^8 (mid dose) or 1.5 x 10^9 (high dose) viral particles/kg of either an early generation vector (Ad5Null) or a "gutless" vector with all viral genes removed (AVGNull). Serum II-6 levels were measured during the first week after dosing. Selected clinical pathological parameters were evaluated on days 2, 8, 15, 29, 45, and 57. Histopathology was evaluated at day 8 and 57. Dramatic increases (200 fold) in serum IL-6 levels were seen in high dose mice 6 hours after receiving either vector. Significant elevations in serum liver enzymes were seen in high dose mice with either vector on day 2, with a decrease in platelets observed in these animals at day 4. Values returned to normal by the termination of the study. Morphological changes were present following treatment with either vector and included hepatocellular apoptosis, extramedullary hematopoiesis, activation of stellate cells and increased extracellular matrix production. Hepatic fibrosis (as measured by sirius red index) in both sexes was not significantly different from controls following treatment with the gutless vector, but significant differences were seen in Av5Null-treated animals. In summary, liver changes produced by the gutless vector were less severe than those due to Ad5Null. Since serum liver enzyme measurements did not reveal these vector-induced hepatic changes, studies are currently underway to determine if serum collagen and alpha-GST levels can be used as markers for early detection of hepatic injury and fibrosis.

1599 BIODISTRIBUTION STUDIES AS PART OF THE PRECLINICAL TOXICOLOGICAL ASSESSMENT OF CANARYPOX GENE TRANSFER VECTORS.

M. Christ1, L. Bonnet2, F. Roché3, J. C. Golffier1, E. Duperray2, E. Fiesse-grompe1, and E. Vencl1. 1IMDS Pharma Services, Lyon, France and 2Aventis Pasteur, Marcy L'Etoile, France.

One critical point in the non-clinical safety evaluation of gene transfer products is the evaluation of the distribution of the exogenous genetic material after administration. Therefore, biodistribution studies are requested by the authorities in the early development phases. The goals of vector biodistribution studies are: (1) to assess potential dissemination of the vector genome with the potential risk of transmission to the offspring and (2) to assess dissemination to non-target tissues, providing information about the potential target organs for toxicity, which helps with the design and conduct of toxicology studies. As part of a toxicology study, we evaluated the biodistribution of the canarypox virus DNA, ALVAC-GP-gp100 mod vaccine, by nested qualitative PCR in Cynomolgus monkey tissue following repeated administrations by the intranasal or subcutaneous route. Forty days after the last ALVAC injection, ALVAC(2)-gp100 mod DNA sequences were found by PCR in the injected inguinal lymph node in 4 out of 4 intranodale-injected animals, and in the right axillary lymph node (draining the last injection site) in one of 4 subcutaneous-injected animals. There were no positive PCR results in any other organs, including gonads. These results complete those from a genetic study in the rat with an vector (a ALVAC vector (no inserted gene administered by 3 different routes (intravenous, subcutaneous and intramuscular). This genetic study included several sacrifice time points and employed a quantitative PCR method. This strategy, employing an extensive analysis of the vector itself without any inserted genes together with PCR data on the candidate product as part of the pivotal toxicology study, not only complies with both US and European requirements but also represents the most rational approach in terms of cost and analysis.
LYSOSOMAL-STORAGE DISORDER INDUCED BY ELMIRON IN RATS AND MICE.

A. Nykä, J. B. Nold, J. D. Johnson and K. Abbö. Laboratory Experimental Pathology, NIEHS, Research Triangle Park, NC. Pathology Associate, Durham, NC. Battelle Columbus Laboratories, Columbus, OH and Environmental Toxicology Program, NIEHS, Research Triangle Park, NC.

Elmiron, a highly sulfated, semi-synthetic pentose polysaccharide with properties similar to heparin, is used for the treatment of intestinal cystitis. Thirteen-week gavage studies were conducted by administering the drug in deionized water to F344/N rats and B6C3Fl mice once daily, 5 days per week for up to 13 consecutive weeks. At doses of 0, 63, 125, 250, 500, and 1000 mg/kg body weight. No significant drug-related effects were observed in body weight, survival, and clinical and gross necropsy results. Significant organ weight changes were seen in the liver, lung, and spleen of both species and the kidney of rats, mainly in groups treated with 250 mg/kg/day and above. Hematological analysis indicated increases for both species in the white blood cell and lymphocyte counts. Sites of toxicity identified histopathologically were the rectum, liver, mesenterium, and mesentery of the lymph nodes (both species), spleen (mice only), and lungs and kidneys (rats only). Lesions consisted mainly of infiltration into multiple tissues of vacuolated histiocytes, histochemical investigation indicated the presence of neutral and acidic mucins and lipoprotein material within the vacuoles. Transmission electron microscopy identified these vacuoles as lysosomal structures that exhibited a variety of contents. On the basis of our findings, we propose that Elmiron was absorbed through the focally disrupted rectal mucosa, accumulated in the lamina propria, phagocytized, and then transported into the lymphatics and blood to the various organs manifesting histiocytic infiltration. The cytoplasmic membrane-bound structures within macrophages are lysosomes containing membranous material of cellular origin and perhaps, remnants of the phagocytized test material, Elmiron.

A FRACTURE HEALING STUDY OF PRINOMASTAT™ IN THE SKELLETALLY MATURE SPRAGUE-DAWLEY RAT.


*Toxicology, CBTR, Sennville, PQ, Canada and **Fisher Global R&D/Augment, La Jolla, CA.

This study was designed to investigate the effect of prinomastat (a matrix metalloproteinase inhibitor) on fracture healing in the rat, during twice daily oral administration at dose levels of 2.0, 100 and 250 mg/kg/day for a minimum of 3 or 6 consecutive weeks, commencing the day following surgical induction of a closed femoral fracture with intramedullary pinning. Selected endpoints (histopathology, biomechanical testing, ex vivo peripheral quantitative computed tomography (pQCT) and ex vivo dual energy X-ray absorptiometry (DXA)) provided an assessment of effects on the reparative and modelling/remodelling phases of the healing process. Surgery involved a paraarticular incision into the joint capsule of anesthetized rats, lateral retraction of the parietal ligament and insertion of an intramedullary Kirschner wire into the femur such that knee joint mobility was unaffected. Following wound closure, a closed transverse fracture of the femoral diaphysis was created using a blunt guillotine driven by a dropped weight. Radiographs were taken following surgery. Minimal comminution and angulation of the intramedullary pin was permitted. Prinomastat was well tolerated, with no adverse toxicological effects, although a reduction in body weight gain was noted. Treatment resulted in reduced biomechanical strength associated with reduced callus formation, evidenced by a reduction in callus area and minimal content as determined by DXA and/or pQCT. Histopathological data suggested prinomastat delayed callus formation/remodelling in rats with an experimentally induced closed fracture. These data suggest that prinomastat delays fracture healing in rats, as evidenced by a reduced callus and biomechanical properties at Week 3 and by persistence of callus at Week 6, however, prinomastat does not completely inhibit the healing process.

POSTPRANDIAL SERUM CALCIUM IS A SUBROGATE MARKER FOR CHANGES IN INTESTINAL CALCIUM ABSORPTION INDUCED BY THE VITAMIN D ANALOG RO 65-2299 IN THE DOG.


Calcium-related toxicity, typical for the class of vitamin D (ViD) analogs, is characterized by increased intestinal calcium (Ca) absorption accompanied by increased renal Ca excretion and at an advanced stage of disturbed Ca homeostasis, hypercalcemia. While urinary Ca and serum Ca concentrations can be easily measured by standard methods, quantification of intestinal Ca absorption requires specialized methods not normally applied in routine studies. In toxicity studies with the ViD analog Ro 65-2299, we established a simple indirect method to identify changes in intestinal Ca absorption in the dog. The animals were adapted to a defined amount of feed given during one hour period in the morning. Ro 65-2299 was administered daily by the oral route during this hour. After at least 5 days treatment, blood was collected before and at 6-8 time points within 6-7 hours after dosing. We demonstrated a dose-dependent effect on the increase of serum Ca concentration during the first few hours after feeding. This was well correlated with Ca-related toxicity observed in subchronic 4-week and 13-week toxicity studies. At non-toxic doses up to 100 mg/kg/day, the postprandial serum Ca concentrations did not exceed the normal range of 2.4-3.0 mmol/L. At 200 mg/kg/day the postprandial Ca level rose slightly above the normal range in some animals but returned to normal before the next day feeding. The 200 mg/kg/day caused only a transient hypercalcemia only after 5 to 8 weeks of treatment. At higher doses (300 and 400 mg/kg/day over 5 days; 250 mg/kg/day over 3 weeks) postprandial calcium levels markedly exceeded the normal range, which corresponded with severe hypercalcemia and hypercalciuria. On the basis of these findings, the test product and GT2 analogs must be evaluated with Ca-related toxicity in mind.

ABSENCE OF TOXICOLOGICAL EFFECTS FROM THE IMMUNOLOGICAL ADJUVANT MONOPHOSPHORYL LIPID A (MPL®).

P. Bockisch, D. Richardson*, G. Elliott* and A. W. Wheeler*.

*CareUS Laboratories Ltd, Harrington, United Kingdom, **Wellcome, United Kingdom and *CareUS Corporation, Hamilton, NI.

The addition of the immunostimulatory adjuvant monophosphoryl lipid A (MPL®), a detoxified bacterial lipopolysaccharide, to a range of conventional vaccine (e.g. malaria, herpes, hepatitis) and allergy vaccine (e.g. grass pollen) materials is the subject of ongoing clinical trials. However, despite this use, there are no robust published data on the nonclinical safety assessment of MPL®. Thus a series of preclinical studies have been performed with the material to evaluate potential toxicological safety issues. An intravenous cardiovascular/respiratory function assessment in anaesthetized dog (at up to 100 µg/kg/day) showed no adverse effects. Repeat dose subcutaneous or intravenous investigations in the rat (for up to 4 weeks and at 250 and 2500 µg/kg/day), rabbit (once weekly for 8 weeks at up to 200 µg/kg/occasion) and dog (for up to 2 weeks and at up to 1200 µg/kg/day) showed no unexplained toxicity findings. Expected immunostimulatory effects and/or signs of toxicity associated with over-stimulation of the immune system (notably increase spleen and white cell activity) were seen in the rat and dog. Embryo-fetal and peri-natal subcutaneous reproduction toxicity studies in rat and rabbit (at up to 100 µg/kg/day) showed no adverse effects. Genotoxicity studies (Ames test and chromosomal aberration assay) were negative. Overall, the lack of findings of toxicological concern support the hypothesis that MPL® is a safe adjuvant for human use.

ORAL MALE FERTILITY STUDY IN RATS.


SB-242235 is a selective p38 protein kinase inhibitor which suppresses pro-inflammatory cytokine biosynthesis. The purpose of this study was to evaluate the potential effects of SB-242235 when administered orally by gavage to male rats, on mating, fertility and gonadal function. Male Sprague-Dawley rats (25/group) were treated with 1% methylcellulose or 3, 10 or 100 mg/kg/day of SB-242235 for 85 to 89 days. On days 15 to 22 and on days 71 to 78, the males were cohabitated with untreated, virgin female (F0) rats. Mated females were killed on day 20 postcoitus. Drug-related mortality (1 of 25 males) and clinical signs (salivation, stereotypic, kneading behavior) were evident at 100 mg/kg/day. Overall, body weight gain for males was reduced (13%, 19%, 46%, at 3, 10, 100 mg/kg/day, respectively) as was food consumption (up to 8%, 22% at 10, 100 mg/kg/day, respectively). There was no effect on pregnancy following mating on days 15 to 22, but pregnancy rate was significantly decreased ex-ante feeding (<4% to 5%) for males given 100 mg/kg/day and mated on days 71 to 78. At 100 mg/kg/day, decreases were evident in weights of the epididymides (left epididymis, intact (22%), right caput and corpus (10%) and right cauda epididymis (36%), and ventral prostate (34%), number of spermatozoa/gram testis (25%) and sperm/granuloid cauda epididymis (52%). There was a decreased (46%) control level of spermatozoa in the lumen of the cauda epididymis. In conclusion, SB-242235 produced mortality at 100 mg/kg/day and overt toxicity at all doses. Reproductive toxicity was demonstrated by reduced organ weights (epididymides and ventral prostate), impaired gonadal function (reduced spermagenesis and sperm motility) and infertility (reduced number of litters sired) among males given 100 mg/kg/day for 10 weeks.
1605 CYCLOSPORIN AND RITONAVIR ARE POTENT P-GP INHIBITORS IN VITRO.

J. S. Moffit, R. J. Mineles, J. J. Xu and D. E. Amacher, DSCGEG, Pfizer, Groton, CT.

In humans the multidrug resistance (MDR-1) gene codes p-glycoprotein (P-gp). As an ATP-dependent export pump, P-gp is involved in the transport of hydrophobic, neutral or cationic xeno- and toxins in a variety of tissues. In addition, the sister of P-glycoprotein (SFGP) is the major canalicular bile salt export pump of mammalian liver. Certain drugs, as for example, cyclosporine (CSA), have been shown to inhibit both P-gp and SFGP, which can result in an impairment of bile flow (cholestasis). Protein trafficking from Golgi to the apical plasma membrane is an important step in the maturation of P-gp. In this study, we compared chrysin, tironavir, progesterone, prazosin, estradiol-17beta-glucuronide, CSA, and wortmannin, as inhibitors of P-gp in two cell line systems. Competition with the efflux of a fluorescent P-gp probe, calcine. The inhibition of P-gp efflux function was investigated following drug treatment in a cell-based assay. The assay compared Madin-Darby canine kidney (MDCK) cells and MDCK cells over-expressing the transfected human MDR1 gene. In the absence of transport inhibition, it has been previously shown that intracellular retention of calcine is high in MDCK cells but low in the MDR1 cells. As a result, we observed inhibition of P-gp efflux function (i.e., increased intracellular calcine fluorescence) by CSA and ritonavir. Our results provide a mechanism for clinically observed effects of CSA and ritonavir.

1606 MISOPROSTOL HAS POTENT IN VITRO TUMORICIDAL ACTIVITY THAT IS INDEPENDENT OF ITS PROSTANOID ACTIVITY.

P. F. Wright1, D. M. Cheah3 and D. R. Haynes3.1 Key Center for Toxicology, RMIT-University, Melbourne, Australia and2 Dept Pathology, University of Adelaide, Adelaide, Australia. Sponsor: D. Di Marco.

Misoprostol (MP) was originally developed as a synthetic prostaglandin (PG) agonist for treating gastritis/ulcers caused by non-steroidal anti-inflammatory drugs. As part of our research program on the immunonodulatory effects of prostanooid compounds, we investigated the effects of MP on the growth and viability of immuno-related tumour cell lines. Mouse leukaemia P388 cells were exposed in vitro to MP over 48 hours, and tumoricidal activity was measured by changes in cell number and viability (via trypan blue exclusion). MP was potent tumoricidally to P388 leukaemia cells, with a 50% inhibitory concentration (IC50) of 100 nM, exhibiting a similar dose-response curve as that reported for MP's immunosuppression of mouse thymocyte proliferation. In contrast, PGE, (the mammalian PGF form and PGF receptor agonist) and dibynzyl cyclic AMP (a stable membrane-permeable analog of the intracellular PG messenger, cAMP), had minimal effects on P388 cells, even when used at 0.1 nM, which is orders of magnitude greater than the IC50 values for their immunosuppressive effects. Furthermore, MP was equally cytotoxic to the adriamycin-resistant subline of P388 (P388/DADR), and was generally more toxic to immune-related tumour cell lines, with the following order of potency: P388 = P388/DADR > mouse lymphoma (Yac-1) > mouse mastocytoma (P815) > mouse fibrosarcoma (Wehi-164). This study indicates that MP is potent cytotoxicity to leukaemia cells, in a manner that is independent of both its prostanooid activity and the mechanisms involved with tumour resistance to adriamycin. These findings have important implications for the potential use of MP as a selective chemotherapy of immune-related tumours. D R Haynes, M W Whitehouse and B Vernon-Reeves (1992) Immunology, 76: 251-257.

1607 AN INTEGRATED APPROACH TO HAZARD EVALUATION FOR WORKER SAFETY ASSESSMENT OF PHARMACEUTICALS AND SYNTHETIC INTERMEDIATES.

M. J. O'Keir3, C. W. Seaman2 and E. J. Guerriero1.1 GlassmanSmithKline, Research Triangle Park, NC, 2GlassmanSmithKline, Westlron, Herts., United Kingdom and 3GlassmanSmithKline, King of Prussia, PA.

Workplaces in the pharmaceutical industry are unique — hazards may be posed both by synthetic chemistry intermediates and the biologically active substances (drugs) created as products. Effective occupational health programs in the pharma industry rely on substance hazard profiles. These are used to identify appropriate containment and protective strategies and are created updated to support the process of drug development. However, hazard characterization is constrained by cost containment efforts, ethical considerations in use of laboratory animals, and recognition of high rates of attrition of substances in development. We have developed an animal screening, toxicology approach for hazard characterization of drugs and intermediates aligned with the development cycle. An initial tier includes physical-chemical characterization (pH/particle coefficient), computerized QSAR (DEREK), in vivo assays assessing skin and eye irritant potential (transcutaneous electrical resistance (TER) and rabbit enucleated eye (REEt) tests), high-throughput mutagenicity assays (SOSumu, HTFT, COMET) and in vitro cytotoxicity tests (predicting acute lethality). Screening of pharmaceuticals occurs earlier in the development cycle than that for intermediates, accounting for changes in the identity of intermediates as syntheses are scaled up for manufacture. A second tier of evaluation involves the local lymph node assay (LLNA) and conventional assays for genotoxic activity (Ames, 15178yr-1). The initial tier is in vitro, in vivo, and ex vivo screens aid by eliminating substances causing positive results from subsequent in vivo evaluation for acute oral toxicity, dermal/ocular irritation, dermal sensitization, and potential target organ toxicity (final tier). Benefits of this approach include support for data-based judgments regarding substance hazards and required labeling at appropriate points in development, and the inherent potential for minimizing extensive in vivo testing.

1608 NEUROTOXICOLOGY OF BLOOD-BRAIN BARRIER AND BLOOD-CSF BARRIER: STRUCTURAL, FUNCTIONAL, AND PHYSIOLOGICAL CONSIDERATIONS.

W. Zheng1 and M. A. Neher2. 1Environmental Health Sciences, Columbia University, New York, NY and 2Physiology & Pharmacology, Wake Forest University, Winston-Salem, NC.

To enter the brain parenchyma chemicals have to cross the brain barrier systems that safeguard the milieu of the central nervous system (CNS). The barrier that separates the systemic circulation from the brain's interstitial fluid is defined as the blood-brain barrier (BBB), while the one that separates the systemic circulation from the cerebrospinal fluid (CSF) is named blood-CSF barrier (BCB). Though ubiquitous, certain areas of the brain, referred to as the circumventricular areas are void of the restrictive properties that characterize the BBB. Research in the past decade suggests that beyond the well-defined role at the BBB, the brain barriers actively participate in brain ontogeny and development, neuroendocrine regulation, drug efflux and metabolism, aging processes, chemical-induced neurotoxics, and the etiology of a number of neurodegenerative diseases. For example, the interaction of manganese (Mn) with iron (Fe) metabolism at brain barrier interfaces alters the cerebral homeostasis of Fe, which may contribute to Mn-induced neurodegenerative disorder. Exposure to organophosphate insecticides impairs the BBB, leading to neurotoxic consequences. Metabolism and/or efflux of drugs and endogenous molecules at the barriers are not only critical for an effective drug therapy, but also essential in preventing CNS side effects. Cumulative evidence also suggests that the BBB, by purifying in the development of cerebral amyloids, plays an important role in the etiology of Alzheimer's disease. Thus, the investigation of transport, metabolism, and molecular interaction at the brain barrier systems has become a new focus in neurotoxicological research. This symposium will provide comprehensive reviews of the cutting edge research in this area, will discuss the use of new technologies in brain barrier studies, and will explore the brain barriers as the new frontier in drug development, toxicity evaluation, and neurodegenerative disorder research.

1609 BRAIN BARRIERS TOWARDS ORGANIC CHEMICALS: DRUG EFFLUX, BIOTRANSFORMATION AND MULTIDRUG RESISTANCE.


The maintenance of brain homeostasis is achieved by 1) the specific properties of the blood-brain interface which control the exchange processes between the blood and the brain, i.e., the cerebral capillaries, the choroidal plexuses (CN), and the meningeal cistern; and 2) a unique cerebrospinal fluid (CSF) circulatory system in the central nervous system (CNS). These specific brain features protect the brain from exposure to different blood-borne drugs and toxins, and regulate the cerebral clearance of neurotoxins. The tight junctions that seal both the endothelial cells of the parenchymal capillaries and the choroidal epithelial cells restrict the entry of polar compounds in the CNS. Brain efflux mediated by transporters such as the P-glycoprotein at the blood-brain barrier and members of the oatp/organic anion-cupporting polypeptide and OAT/organic anion transport families at the CP epithelium, also limits the cerebral bioavailability of various lipophilic, amphoteric or anionic compounds. Several drug metabolizing and antioxidant enzymes are present at both the blood-brain and blood-CSF barriers. In particular, CP appear to be a major site of detoxification in the brain. The use of an in vitro model of this blood-CSF interface allows to demonstrate the existence of an effective metabolic barrier toward neurotoxins at the choroid plexus. This conceptual metabolic barrier is dependent upon the intracellular level of cofactors, such as glutathione, and results from active conjugation processes coupled to a polarized basolateral (i.e., blood-facing) efflux of the conjugated metabolites via a multidrug-resistant
associated efflux pump. Reactive compounds, however, can also be generated at these interfaces by metabolic activation, a process that could lead to a disruption of brain homeostasis, should the antioxidative capacity of the interfaces be overflown.

**1610 BLOOD-BRAIN BARRIER TRANSPORT OF METALS: IMPLICATIONS IN CNS HOMEOSTASIS OF ESSENTIAL AND NONESSENTIAL METALS.**

M. Aschner, Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC.

This lecture will highlight the transport of various metals (e.g., manganese and mercury) across the blood-brain barrier (BBB). It will emphasize the mechanisms of transport and address the means of both essential (manganese) and nonessential (mercury) metal transport by the BBB, as the mechanisms of ionic (ion) and molecular (methionine) mimicry, respectively. An important process in the toxicologic outcome of exposure to metals is their transport from plasma into the brain across the capillary endothelial cells that comprise the BBB. In order to cross this barrier, metal complexes must be either highly lipophilic, or possess affinity for specific carrier-mediated transport systems within the endothelial cell plasma membrane. Little is known about manganese and mercury transport. The objectives of this lecture are, therefore, to identify the transport mechanism(s) of mercury and manganese, under the assumption that increased rate of metals transport across the BBB is reflected in elevated CNS concentrations and symptoms due to increased neurotoxicity. Emphasis will be directed to experimental results derived from both in vivo and in vitro studies, as well as elaborating on the advantages and disadvantages of current methodologies for the assessment of transport kinetics of these metals, such as tissue culture techniques and in vivo microdialysis. (Supported by Public Health Service Grant ES07331 and ES10565)

**1611 INTERACTIONS BETWEEN TOXICANTS AND TIGHT BARRIERS.**

L.B. Brentler1, D.J. Bannon2, L.B. Olivi2, and J. Cheong2. 1Neurology, Kennedy Krieger Institute, Baltimore, MD and 2Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.

Tight barriers such as those formed by epithelial and brain endothelial cells prevent the passage of large molecules or those carrying charges from entering brain, thus providing protection for neural cells against toxins. Nonetheless, toxicants are capable of passing through barriers. Some toxicants have been shown to increase nonselective permeability by compromising the integrity of the blood brain barrier (BBB). A class of transporters that mediate uptake of metals, for example Pb, are the anion exchangers (AE). Because AE is highly expressed by choroid epithelium, it may explain why lead (Pb) is readily taken into the choroid plexus. However, physiological and anatomical studies have suggested that AE are unlikely involved in the transport of Pb through the intestinal barrier and the BBB. Instead, the major intestinal iron transporter, i.e., divalent metal transporter 1 (DMT1) which has been shown to transport a broad range of metals, may be involved. Overexpression of DMT1 in yeast by results in an increased uptake of Pb, whereas knockdown of DMT1 in intestinal epithelial cells results in a decreased uptake of Cd, suggesting that DMT1 mediates the transport of Pb and Cd. Interestingly, DMT1 requires H+ for its function and thus is not likely to be involved in the transport of Pb from the blood across the BBB. Pb at higher concentrations do not require a transporter because it induces a breakdown in the BBB. Organophosphate insecticides also increase permeability of toxicants by inducing a similar breakdown in the BBB. In summary, tight barriers express specific transporters for nutrients that can also transport toxic metals. Additionally, toxicants can increase permeability by compromising the integrity of the tight barriers.

**1612 CONTRIBUTIONS OF BRAIN BARRIERS IN NEURODEGENERATIVE DISORDERS AND CHEMICAL-INDUCED NEUROTOXICITIES.**

W. Zheng, Environmental Health Sciences, Columbia University, New York, NY.

Normal neurological function relies on the delicate chemical balance among neurons, neuroglia and their associated synaptic connections. The damaging effect of a chemical to the barriers can contribute substantially to chemical-induced neurotoxicity. Mechanically, some chemicals directly disrupt barrier structure and increase its permeability, perturbing the chemical milieu and allowing for unregulated materials to come into contact with brain parenchyma. Other chemicals may not directly alter the morphology of the barriers, but rather impair barriers' regulatory function, bringing about neurotoxicity due to disordered cerebral homeostasis. Furthermore, certain chemicals prior to their entrance to the cerebral compartment are bio-activated or bio-deactivated by metabolizing enzymes present in brain barriers. Recent evidence also suggests that the structural and functional integrity of brain barriers is important in the etiology of Alzheimer's disease. The aging of the cerebral vascular structure, the ability of barriers to transport extracellular beta-amyloid, and the production of beta-amyloid solubilizing macromolecules by the barriers, are amongst the possible contributory factors under considerable scrutiny, although the role of chemical exposure in disordered barriers of Alzheimer's remains poorly understood. In addition, the dysfunction in iron regulation at brain barriers caused by manganese (Mn) exposure has been suggested to underlie a Mn-induced Parkinsonism-like syndrome. This lecture examines the current understanding of the role of brain barriers in CNS disorders by focusing on chemical-induced neurotoxicity. The perspectives for future research needs will also be discussed.

**1613 NIEHS SYMPOSIUM: ENVIRONMENTAL INFLUENCES ON CHILDHOOD ASTHMA.**

K. M. Olden, NIEHS, Research Triangle Park, NC.

Childhood asthma is the subject of the symposium. This disease was chosen because of the strong link to environmental exposure, the genetic-environment interactions, and because it represents an example of how basic research can be translated into intervention and prevention programs. In addition, childhood asthma is a chronic lung disorder of enormous public health importance that disproportionately affects minorities and persons of low socioeconomic status. It is the most frequent cause of pediatric emergency room use and hospital admissions and is the leading cause of school absences. Despite improvements in diagnosis and management, and an increased understanding of the epidemiology, immunology, and biology of the disease, asthma prevalence, morbidity, and mortality have progressively increased over the past 15 years. To reduce the incidence of childhood asthma a multidisciplinary approach to the research effort is necessary. This symposium will include presentations that span research in epidemiology, molecular mechanisms, gene-environment interactions, and environmental agents related to the disease, and intervention and prevention of childhood asthma.

**1614 ENVIRONMENTAL INFLUENCES ON CHILDHOOD ASTHMA.**

F. D. Martinez, Arizona Respiratory Center, University of Arizona, Tucson, AZ. Sponsor: K. Olden.

Significant advances have been made during the last ten years in our understanding of the environmental factors that may protect against or predispose to the development of asthma. The finding that most cases of asthma begin during the first years of life has focused the attention of many researchers to the factors that may influence the risk for asthma in these early years of development. The study of risk factors for asthma is complicated by the heterogeneous nature of the disease. Recurrent airway obstruction is the final common pathway for at least three different syndromes, which co-exist during infancy and childhood. "Transient infant wheezing" is confined to the first 2-3 years of life and is benign in course and mainly associated with maternal smoking during pregnancy. "Non-atopic wheezing," in a typical disease of infants and toddlers but may also persist up to the school years. It is characterized by attacks or wheezing mainly associated with wintertime infections. "Atopic asthma" is least frequent during the early years but becomes the most prevalent form of asthma by early adolescence. It is associated with chronic symptoms, deficits in lung function, and persistence of symptoms into the adult years. It now appears that, whereas early microbial burden is a risk factor for transient infant wheezing and non-atopic asthma, several studies have shown that such burden is a protective factor against the development of atopic asthma. The mechanism by which microbial burden in early life protects against the development of atopic asthma is beginning to be understood. Exposure to external "danger" signals that interact with the innate immune system may be necessary for the development of a mature, balanced immune response that may last a lifetime. Lack of such microbial burden at crucial times at the development of the immune system may select certain forms of immune responsiveness that are associated with allergic sensitization, airway hyperresponsiveness, and chronic asthma systems in susceptible individuals.

**1615 ENVIRONMENTAL AGENTS RELATED TO CHILDHOOD ASTHMA.**


Prior studies indicate that exposure to a variety of indoor allergens, such as those that derive from house dust mites and cockroaches, are important risk factors for the development of allergy and asthma in children. Moreover, recent studies indicate that bacterial endotoxins may be an important environmental factor in asthma.
pathogenesis and that exposure to both allergens and endotoxin may cause a more severe inflammatory response than exposure to either stimulus alone. The National Allergen Survey is a descriptive study of allergen levels and endotoxin in indoor and outdoor environments. The survey was conducted from 1998-1999 using a population-based, multi-stage area probability design which has yielded a sample that is representative of diverse geographic regions, socioeconomic groups and housing types. It encompasses 831 homes in 75 primary sampling units located throughout the country. The major endpoints are measurements of dust mite (Der f 1, Der p 1), cockroach (Blatta g 1), cat (Fel d 1), dog (Can f 1), rodent (Mus m 1, Rat n 1) and fungal (Alternaria) allergen levels, and endotoxin in dust samples collected from an array of sites in the surveyed homes. This presentation will: (a) provide a brief overview of previous studies which demonstrate a relationship between indoor allergen/endotoxin exposure and asthma/allergy; (b) review preliminary data from the National Allergen Survey which provide current estimates of indoor allergen/endotoxin exposure in the US housing stock; and (c) discuss regional, socioeconomic, and housing factors that influence indoor allergen/endotoxin exposure.

1616 CELLULAR AND MOLECULAR MECHANISMS OF ASTHMA PATHOGENESIS.

M. Wills-Karp. Division of Immunobiology, Children's Hospital Medical Center, Cincinnati, OH. Sponsor: D. Brown.

Asthma is characterized by variable airflow obstruction, airway hyperresponsiveness and airway inflammation. The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall with mast cells, lymphocytes and eosinophils. Although asthma is multifactorial in origin, recent advances suggest that asthma is an immunological disease with a prominent role for T lymphocytes in the pathogenesis. As such, CD4+ T cells producing a Th2 pattern of cytokines (IL-4, IL-13, IL-5) have been hypothesized to play a pivotal role in the pathogenesis of this disease. The paradigmatic Th2 cytokines, IL-4 and IL-5, had been thought to be the primary regulators of the allergic response. However, recent studies from my group have shown that IL-13 is likely the central mediator of the effector phase of allergic responses. Specifically, we have observed the blockade of IL-13 at the skin, antigen challenge in antigen-sensitized mice via administration of a IL-13Rα2-βg which only binds IL-13, ablated antigen induced AHR. Furthermore, delivery of the recombinant cytokine to the lungs of naive animals reproduced the entire allergic phenotype (i.e. AHR, eosinophilia, mucus hyperplasia). A role for IL-13 in human asthma has been suggested by the demonstration that IL-13 mRNA and protein levels are elevated in the lungs of asthmatics as compared to normal individuals. Although the exact mechanisms by which IL-13 induces AHR are currently not known, IL-13 has effects on a number of effector cells thought to be important in asthma including mast cells, B cells, eosinophils, airway epithelial cells, and smooth muscle cells. Grung et al., 1998 have shown that T and B cells are not necessary for IL-13-induced AHR. Recent findings from my laboratory suggest that IL-5 and eosinophils are also not necessary for the effects of IL-13 on airway reactivity. As IL-13 receptors are expressed on airway smooth muscle cells, IL-13 may have direct effects on airway smooth muscle contractile function. Determination of the exact mechanisms by which IL-13 induces airway hyperresponsiveness awaits further studies.

1617 GENE-ENVIRONMENT INTERACTIONS IN CHILDHOOD ASTHMA.

S. Kleeberger, Environmental Health Sciences, Johns Hopkins University, School of Public Health, Baltimore, MD.

The prevalence of asthma morbidity and mortality has increased in industrialized countries at an alarming high rate for the last 20 years. The rates are disproportionately high in urban environments and in children, but the causes are unknown. It has become increasing clear that genetic background is an important determinant. Inheritance patterns of asthma indicate that it is a complex, multifactorial disease. The complexity stems partly from the number of subphenotypes that characterize the disease, including airways hyperresponsiveness, inflammation, and allergic status. For each subphenotype, there may be a gene or set of genes that confer susceptibility or predisposition, and these genes interact to produce the asthma phenotype(s). Genome-wide searches for linkage of asthma subphenotypes have revealed a number of quantitative trait loci, each of which has potential candidate genes for the phenotype in question. Further, family and population studies have identified linkage of specific phenotypes with candidate genes. However, genetic background alone cannot account for the increasing asthma prevalence. The expansion of the disease or its subphenotypes is likely dependent on environmental factors. That is, the relative risk of asthma for a specific genotype or combination of genes may be a function of the frequency of exposure to the environmental agent, the strength of interaction between the genotype and the agent, and the specificity of the environmental effect with respect to the genotype. Potentially important environmental exposures for asthma include indoor air pollutants (NO2, tobacco smoke), outdoor air pollutants (ozon, PM2.5 and ultraviolet, NO2, and SO2), and allergens such as house dust mite, cockroach, pollen, and cat dander. Assessment of exposure to these agents is critical to evaluating any relationship between specific exposure and the disease process, and it is clear that the environment is a potent mediator of the disease process.

1618 A COMMUNITY-BASED PARTICIPATORY RESEARCH APPROACH TO REDUCING ENVIRONMENTAL TRIGGERS TO CHILDHOOD ASTHMA IN DETROIT.


The Michigan Center for the Environment and Children's Health (MCECH), is a member of the NIH/EPA Children's Environmental Health and Disease Prevention, MCECH's research program focuses on: 1) determining the effects of allergen-induced local, excessive production of chemokines on the respiratory system; 2) assessing the possible interaction of effects of outdoor and indoor air quality on exacerbation of asthma in children; and 3) household and neighborhood level interventions focused on reduction of environmental triggers for asthma among children in the southwest and east side of Detroit. The latter two projects have been combined to form Community Action Against Asthma (CAAA). A unique feature of the CAAA project is the use of a community-based participatory research approach. CAAA's household intervention employs Community Environmental Specialists (CES) who visit the homes of intervention participants using an individualized plan that has been developed jointly with the caregiver. This plan records the results of the child's allergy skin test, a household dust sample, the caregiver's responses on a baseline survey and a household environmental assessment tool. Through this plan, the CES's are able to tailor the activities in their visit (e.g., education on asthma and indoor environmental triggers and social support to improve; e.g., vacuum cleaners, mattress covers, cleaning supplies); integrated pest management services and referrals to social service/health agencies to emphasize those changes likely to impact the child's asthma symptoms.

1619 IS THERE REPRODUCTIVE RISK ASSOCIATED WITH EXPOSURE TO DISINFECTION BY-PRODUCTS OF DRINKING WATER?

G. R. Kleinheister, Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.

The Safe Drinking Water Act requires the US Environmental Protection Agency to establish national drinking water standards that protect the health of the 250 million people who get their drinking water from public water systems. Today there is considerable interest in the part of Stakeholders (i.e., Congress, water industry, environmental groups) in the potential adverse reproductive and developmental outcomes associated with exposure to drinking water disinfection by-products (DBPs). Epidemiological and toxicological data suggest an association between elevated DBPs and adverse reproductive outcomes such as spontaneous abortion, stillbirth, and developmental anomalies. While there are still insufficient epidemiological data to substantiate causality, the toxicological data provide some degree of biological plausibility. Of particular interest are emerging toxicology data which establish the potential for DBPs to adversely affect the developing reproductive system, indicating that animals with a longer period of reproductive development are more sensitive to DBP-induced effects, and identify novel biomarkers applicable to epidemiology. In addition, proteomic data provided from both developmental and reproductive studies might aid in the elucidation of the mode of action for a chemical by-product formed during the process of drinking water disinfection. During summer 2002 the Federal Advisory Committee will cast its final decision on the existing Stage 2 DBP rule which sets the maximum contaminant level goal for by-products in finished drinking water. Toxicology data demonstrate that sensitive reproductive and developmental endpoints are perturbed by specific disinfection by-products (DBPs). Epidemiological data suggest an association between elevated DBPs and adverse reproductive outcomes such as spontaneous abortion, stillbirth, and developmental anomalies. While there are still insufficient epidemiological data to substantiate causality, the toxicological data provide some degree of biological plausibility. Of particular interest are emerging toxicology data which establish the potential for DBPs to adversely affect the developing reproductive system, indicating that animals with a longer period of reproductive development are more sensitive to DBP-induced effects, and identify novel biomarkers applicable to epidemiology. In addition, proteomic data provided from both developmental and reproductive studies might aid in the elucidation of the mode of action for a chemical by-product formed during the process of drinking water disinfection.
tial health risk. The chemical mixture of DBPs include trihalomethanes, haloacetic acids, and haloacetonitriles/aldehydes. Reproductive endpoints are important potential outcomes of DBP exposure for several reasons: (1) due to the ubiquitous nature of the exposure, small increases in relative risk will have a significant public health impact; (2) the fetus and reproductive system are sensitive to environmental toxicants; and (3) they can be effectively studied because the interval between exposure and outcome is short. The epidemiologic database evaluating this relationship remains sparse. To date, there have been 18 studies published; 9 relied on reported quarterly concentrations of THMs in water supplies serving the subjects' community as the exposure metric. The remainder used a qualitative exposure assessment to estimate associations between water source or method of treatment and risk of adverse reproductive outcome. Several studies found weak associations between exposure to DBPs and adverse reproductive outcomes including spontaneous abortion, stillbirth, low birth weight, intrauterine growth retardation, somatic parameters and developmental anomalies (e.g. heart, oral cleft and central nervous system malformations). Recent studies suggest that the brominated DBPs may be more hazardous. However, the findings are inconsistent and the epidemiologic evidence is insufficient to infer a causal relationship. Additional laboratory and human studies are needed before this issue can be resolved. Future human studies should incorporate improved methods of exposure assessment to reduce misclassification, focus on specific byproducts, especially the brominated species of the trihalomethanes and haloacetic acids, and include additional outcomes such as fertility, fecundity and potential effects on semen markers.

1621 DEVELOPMENTAL CONSEQUENCES OF EXPOSURE TO DISINFECTION BY-PRODUCTS IN ANIMAL MODELS.

E. S. Hunter. Reproductive Toxicology Division, USEPA, Research Triangle Park, NC. Sponsor: G. Klingele.

Disinfection by-products (DBPs) have been associated with a variety of adverse developmental outcomes in epidemiology studies including cardiac defects, neural tube defects, stillbirths, and spontaneous abortions. In toxicology studies, the trihalomethanes (THMs) do not produce high rates of dysmorphogenesis, but selected THMs do cause pregnancy loss, i.e. full-litter resorptions (FLR) in rats. Serum lactogenic hormone (LH) is diminished when bromochloroacetonitrile (BCAN) is administered to Fischer 344 rats during the LH-dependent period of pregnancy. Evidence suggests that perturbation of the endocrine axis may be responsible for FLR. The possibility that BDCA compromises LH responsiveness is also being explored as this has more relevance to human pregnancy maintenance. Among the haloacetic acid (HA) class of DBPs, trichloroacetic (TCA) and dichloroacetic (DCA) each induce heart, eye and kidney defects in rats. Dibromoacetic acid (DBA) produces small abdomens in mice, eye defects in rats and skeletal abnormalities in both species. Bromochloroacetic acid (BCA) exposure results in decreased fetal implants and live fetuses. In the rat, BCA reduced day 6 pup weights, and DBA induced pre- and postnatal loss and reduced pup weight on PND 1 and 6. When compared in mouse whole embryo culture (WEC) the relative potency of the HA s are BCA > DCA > DBA. Dose additivity of DCA, DBA and BCA was observed in rat WEC. Haloacetonitriles, another class of DBPs, induce FLR [dichloroacetonitrile (DCAN) and trichloroacetonitrile (TCAN)], decrease birth weight [chloroacetonitrile (CAN), TCAN], increase postnatal weight gain (CAN, BCAN, DCAN), increase postnatal mortality (CAN, TCAN). While most DBPs produce effects on developmental and neonatal growth, many are only effective at doses that produce maternal toxicity. In vivo, embryos show induction of cell death with little concurrent effect on the cell cycle. Mechanistic studies are focused on HA-induced alteration in signal transduction pathways.

1622 REPRODUCTIVE TOXICITY IN THE MALE RAT FOLLOWING EXPOSURES TO DISUBSTITUTED HALOACETIC ACIDS.

G. R. Klingele. Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.

Over the past decade many studies have demonstrated that disubstituted haloacetic acids present in drinking water as a result of disinfection produce adverse reproductive consequences in the male rat. Epidemiologic studies have shown that 1-2 days alters spermagogenesis in a manner which would indicate alterations in sperm quality, and indeed, fertility of cauda epididymal sperm is significantly compromised. The reduction in fertility is associated with diminutions in proteins in detergent extracts of sperm. One protein, SP22, is highly correlated with resultant fertility. Based on emerging data generated in humans using anti-SP22 antibodies, and future studies of drinking water epidemiology studies may incorporate an assessment of this biomarker. It is interesting that when haloacetic acids span the duration of spermatogenesis (i.e. 70 days), seminol-cell only seminiferous tubules manifest, even 6 months after cessation of exposure. This exacerbation rather than recovery may be significant in species in which spermatogenesis is less efficient (e.g. humans). Evidence that the magnitude of effects increase with duration of exposure suggests that these DBPs have also been provided by assessments of the developing reproductive system. It is now clear that the duration of exposure rather than the window of exposure accounts for significant delays in prepubertal separation. Again, implications are a concern for species with a longer period of reproductive development. Spermatogenesis, fertility (including mating ability), and puberty are each hormone dependent processes and it is these processes that are altered significantly by these haloacetic acids. Recent studies suggest that the brominated DBPs may be more hazardous. However, the findings are inconsistent and the epidemiologic evidence is insufficient to infer a causal relationship. Additional laboratory and human studies are needed before this issue can be resolved. Future human studies should incorporate improved methods of exposure assessment to reduce misclassification, focus on specific byproducts, especially the brominated species of the trihalomethanes and haloacetic acids, and include additional outcomes such as fertility, fecundity and potential effects on semen markers.

1623 THE RABBIT MAY REPRESENT A BETTER ANIMAL MODEL FOR DETERMINING REPRODUCTIVE RISK ASSOCIATED WITH DBPS.

D. N. B. Veramachanid. Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO.

Rabbits have a long quiescent period of reproductive development before puberty mimicking human development better than rodents. Moreover, longitudinal evaluation of endocrine profiles, sexual capacity, and semen quality are feasible in rabbits. These considerations suggest that the rabbit may be a more relevant animal model in evaluating human reproductive health risk. For the study of dibromoacetic acid (DBA), male and female rabbits were exposed to sin drinking water from gestation day 15 through puberty (0, 1.5, and 5 mg/kg). Six ejaculates (one every third day) were collected and semen capacity and seminal quality were determined. Two untreated females were artificially inseminated with a known number of sperm from each experimental male and pregnancy outcome determined. Males in the DBA groups showed reduced sexual interest and ejaculation failures. Rabbits in the 50 mg group that completed ejaculation took more attempts (1.5 to 1 in control) and longer time (50 to 15 sec) to achieve ejaculation; two had no sperm in their ejaculates at least once. The conception rates for male rabbits in 0, 1.5, and 50 mg groups were 85, 55, 65 and 55%, respectively. The individual conception rates of each of the treated groups were significantly lower than that of the control group. Male rats exposed similarly had reduced fertility at 50 mg/kg. Female rabbits in the DBA groups had reduced populations of primordial follicles; numbers were significantly lower in both the 50 and 50 mg/kg groups. Female mice exposed similarly to DBA, were found to have no differences in the populations of ovarian follicles. That sexual function, seminal quality (reflected by lowered fertility even at 1 mg/kg), and primordial follicle populations were more sensitive in rabbits compared to mice and rats, may reflect longer exposure during reproductive development in the rabbit. Thus, the rabbit may be a more useful model for human reproductive health risk assessment than rodents, particularly when cumulative risk is indicated and lowered effects are anticipated.

1624 DO AVAILABLE TOXICOLOGY DATA ON DBPS IMPACT OUR ABILITY TO ASSESS HUMAN REPRODUCTIVE RISK?

R. W. Yl. Center for Life Sciences & Technology, Research Triangle Institute, Research Triangle Park, NC.

The strengths of the recent animal data set are the sensitivity of the endpoints, the characterization of the DBP(s) employed, and the use of exposure during sensitive life stages. There is some concordance of outcomes from the *in vivo* to *in vitro* animal studies, and from *in vivo* animal studies to some epidemiologic studies. The weaknesses of the animal studies are the over reliance on route of administration, exposure levels, exposures to single DBPs rather than mixtures, risks of false positives/negatives (especially in *in vitro* work), and limited gestational exposure duration and endpoints. Epidemiology studies employ large populations and methods to correct for confounders, but there is limited, and nonconcurrence characterization of the DBPs (are surrogate markers). Long-term and longer time (30 to 15 sec) to achieve ejaculation; two had no sperm in their ejaculates at least once. The conception rates for male rabbits in 0, 1.5, and 50 mg groups were 85, 55, 65 and 55%, respectively. The individual conception rates of each of the treated groups were significantly lower than that of the control group. Male rats exposed similarly had reduced fertility at 50 mg/kg. Female rabbits in the DBA groups had reduced populations of primordial follicles; numbers were significantly lower in both the 50 and 50 mg/kg groups. Female mice exposed similarly to DBA, were found to have no differences in the populations of ovarian follicles. That sexual function, seminal quality (reflected by lowered fertility even at 1 mg/kg), and primordial follicle populations were more sensitive in rabbits compared to mice and rats, may reflect longer exposure during reproductive development in the rabbit. Thus, the rabbit may be a more useful model for human reproductive health risk assessment than rodents, particularly when cumulative risk is indicated and lowered effects are anticipated.
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t), better ascertain reproductive outcomes, and control for confounders, etc. All of these studies are expensive, labor intensive, difficult, and of long duration. We must address the costs versus the human health benefits of such efforts. Is it reasonable to generate data which may/may not result in the reduction of MCLGs (maximum contaminant level goals) if cost savings resulting from possible human health benefit cannot be determined? In other words, does the benefit (if any) offset the cost both with respect to research and treatment plant MCLG reduction? There is a hazard: is there sufficient risk?

1625 NON-CLINICAL TESTING OF PHARMACEUTICALS FOR PEDIATRIC USE.

Since the issuance of the Pediatric Final Rule by the FDA in 1998, the importance of adequate testing for the safety and efficacy of pharmaceutical products in children has become clear. The Pediatric Rule defines what products require testing, when pediatric studies are needed (with waiver and referral provisions), and expected interactions with the FDA. The FDA Guidance for Industry for compliance with the Pediatric Rule is expected to provide further clarification of the expectations of the Rule. To support clinical studies of new pharmaceuticals in children, preclinical safety studies must be conducted. Many organ systems (eg., central nervous system, pulmonary, reproductive, immune, etc.) continue to develop postnally, sometimes until adulthood, and develop at different rates in different species. Since standard reproductive and developmental toxicity studies as described by ICH guidelines do not cover direct exposure of offspring during the postnatal and perinatal periods, special juvenile toxicity studies may be necessary. Depending upon the organ system of particular concern, different periods of postnatal exposure, and different animal species, may be most appropriate. When designing juvenile toxicity studies, many practical considerations must be taken into account, such as feasibility of dosing, sampling procedures, and development of new methodology for assessment of targets in young animals. Finally, the FDA experiences in reviewing study designs and results of juvenile toxicity studies will provide insight into the future designs and conduct of these studies.

1626 UNDERSTANDING THE PEDIATRIC RULE: AN INDUSTRY PERSPECTIVE.
L. A. Dostal, Drug Safety Evaluation, Pfizer Global Research & Development, Ann Arbor, MI.

The Pediatric Rule was issued as a regulation effective April 1, 1999 and requires the sponsor of an NDA or marketed product to provide information on the efficacy and safety of the drug product in children. This presentation will describe the requirements of the Rule as it applies to new active ingredients or dosage forms that are used in a large number of pediatric patients, where there is a potential for significant improvement in treatment, or where there is no current therapy available for children. If the sponsor believes that none of these conditions apply, they may request a waiver of the studies, or a deferral of the pediatric assessment until after the NDA is submitted. Often the design of a pediatric plan is complicated by the development of a suitable pediatric formulation and issues of safety of dosing infants and children with new chemicals that are not already demonstrated to be safe and effective in adults. This presentation will cover various strategies and questions that could be considered in the design of an acceptable Pediatric Plan to address the Pediatric Rule.

1627 SPECIES DIFFERENCES IN POSTNATAL DEVELOPMENT OF TARGET ORGAN SYSTEMS.
T. Zoeis, Millstone Biomedical Associates, Frederick, MD.

Animal studies have been used to investigate the response of immature systems for many years. Developmental toxicity studies have traditionally focused on the pre-natal period of growth and maturation. Studies using juvenile animals are currently being designed to investigate developmental issues that occur postnatally in humans. Importantly, not all target organ systems develop during the same timeframe with reference to the time of birth for different species. Selecting the appropriate species and timing of dosing are critical to the success of postnatal developmental studies. A consideration in making this selection is the timeframe in which exposure is expected during the development and maturation of the given test species compared to humans. Differences in timing of growth and maturation between species can be considered using microscopio evaluation for cellular proliferation and differentiation, as in the case of postnatally developing doepl, or by other methods appropriate for assessing organ functional capacity, as in the postnatally developing nervous system. In some cases, the immature systems of children can render the toxicity of a chemical to be lower than that occurring in the adult due to differences in metabolism. On the other hand, children may lack the ability to detoxify a chemical resulting in a higher level of toxicity when compared to adults. Organ systems that undergo major postnatal development or maturation include nervous, skeletal, renal, pulmonary, and reproductive. Examples of drugs that behave differently in adult (mature) and pediatric (immature) organ systems will be presented to illustrate the effects of differences between species and the timing of maturation in the study of postnatal development.

1629 FDA EXPERIENCE WITH NONCLINICAL DATA SUBMITTED TO SUPPORT DRUG TRIALS IN PEDIATRIC PATIENTS.

Pediatric patients may be more susceptible to certain drug toxicities compared to adults, possibly because organ systems could be more vulnerable to insult during development than when fully developed. There are a number of examples of increased susceptibility to drug toxicity in pediatric patients compared to adults. There are also examples of unique toxicities seen in this population that have not been observed in adults. The Center for Drug Evaluation and Research has received reports of nonclinical toxicology studies submitted to support the safety of clinical drug trials in infants and children. Analysis of some of these data sets supports the idea that there can be adverse effects in pediatric patients which might not have been anticipated based on studies in relatively mature animals or adult humans. These observations indicate that nonclinical toxicology studies in immature animals might be valuable in determining the safety of clinical trials in pediatric subjects. Nonclinical toxicology studies have varied in design and the potential implications of these variations will be discussed with respect to pediatric risk issues.

1630 RECENT DIOXIN CONTAMINATION FROM AGENT ORANGE IN RESIDENTS OF A SOUTHERN VIETNAM CITY.
A. S. Becker, L. C. Dai, O. P. Lo, M. P. Weng, J. P. Pang, J. D. Constand, M. Matsuda and V. Duc Tho. University of Texas-Houston School of Public Health, Houston, TX. 'Vietnam Red Cross, Hanoi, Viet Nam,' ERGO Laboratory, Hamburg, Germany. 'National Research Center for Environmental Toxicology, Griffith University, Brisbane, Australia.' Harvard Medical School, Massachusetts General Hospital, Boston, MA, 'Ehime University, Matsuyama City, Japan' and Institute for Environmental Science and Technology, Harris University, Hanoi, Viet Nam.

Marked elevation of dioxin associated with the herbicide Agent Orange was recently found in most blood samples from Vietnamese living in Bien Hoa, a large city in southern Vietnam near Hoi Chi Minh City. This city is located near an airbase used for Agent Orange spray missions between 1962 and 1970. A spill of Agent Orange occurred at this airbase over 30 years prior to blood collection in 1999. Blood samples were collected, and are part of a World Health Organization-certified dioxin laboratory for congenator-specific analysis as part of a Vietnam Red Cross project. Dioxin congeners that contaminated Agent Orange, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), was elevated in the blood of 40 out of 42 (93%)
Bien, Hsia residents. Median TCDD level was 67 parts per trillion (ppt) (Range 2.4 to 413 ppt). A comparison sample from over 100 residents of Hanoi where Agent Orange was not used, had blood TCDD levels of 2 ppt. TCDD contamination was also found in some nearby soil and sediment samples. Persons new to this region and children born after Agent Orange spraying ended also had elevated TCDD levels. This TCDD uptake was recent and occurred decades after spraying ended. We hypothesize that a major route of current and past exposures is from the movement of dioxin from soil into river sediment, then into fish, and from fish consumption into people.

1631 SERUM TCDD LEVELS AND THYROID EFFECTS AMONG US AIR FORCE VETERANS.

M. Pawlik, A. Schecter2, and R. Akhtar.1. University of Texas Houston School of Public Health in Dallas, TX and Spectro Pro Inc., Austin, TX.

We assessed potential health effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) on thyroid function in US Air Force veterans involved in aerial spraying of herbicide Agent Orange during the Vietnam War from 1962 to 1971 (Operation Ranch Hand). We examined associations between serum TCDD level with levels of thyroxin (T4), thyroid stimulating hormone (TSH), triiodothyronin percent uptake (T3% uptake), free thyroxin index (FTI), as well as with incidence of thyroid diseases. Data were available for 1039 Ranch Hands and 1429 comparison veterans categorized into four exposure groups — Comparison, Background, Low, and High. We found consistently elevated levels of TSH in the high exposure group of Ranch Hand over all five medical examinations. We also observed statistically significant dose-response relationship between TSH and TCDD levels in 1982, 1985, 1987, and 1992. The High exposure group Ranch Hands had also a higher risk of having abnormally high TSH levels over all five exams, with 1997 results approaching statistical significance. Overall, the incidence of thyroid diseases was not increased in Ranch Hand veterans. These findings suggest that the highest exposure group of Ranch Hand veterans, TCDD may affect thyroid hormone metabolism and function.

1632 SEQUENCE VARIATION AND PHYLOGENETIC HISTORY OF THE MURINE AH LOCUS.

D. R. Rank1, S. G. Penn, K. Holdon2, C. A. Bradford2, and R. S. Thomas.'

1Aquatics Inc., Sunnyvale, CA and 2McMillan Cancer Research Laboratory, University of Wisconsin, Madison, WI.

The Ah locus encodes for the aryl hydrocarbon receptor (AhR) which plays an important role in toxicological and developmental roles. Sequence variation at this locus was studied in 13 different mouse lines that included 8 laboratory strains, 2 Mus musculus subspecies, and 3 additional Mus species. The data represented the largest study of sequence variation across multiple mouse lines at a single locus (+15.9 kb/mouse line). A total of 2,213 polymorphisms were identified and used to estimate the polymorphism rates and population characteristics among all mouse lines. Among all mouse lines, the average frequency of polymorphisms in the intronic regions was 20.3 SNPs/kb and the average exonic frequency was 14.1 SNPs/kb. Between laboratory strains, the average intronic and exonic frequencies dropped to 5.4 and 2.9 SNPs/kb, respectively. By comparison, polymorphism frequencies were significantly higher at this locus than those observed in the whole genome scans. There were 111 non-synonymous polymorphisms that resulted in 42 different amino acid changes, of which only 10 amino acid changes have been previously identified. Based on the nucleotide sequence, the phylogenetic history of the gene showed mice from the AhRP and AhP alleles in separate branches while mice from the AhR and AhF alleles exhibited a more complex history. Evolutionarily, the AhR protein as a whole appears to be under purifying selective pressure (Kd/Ks = 0.237). Despite significant functional constraint in the basic-helix-loop-helix and PAS domains, ligand binding is not constrained to the high affinity allele, which further supports the role of the AhR in development and its importance beyond the adaptive response to environmental toxins.

1633 TCDD AND THE BIOLOGICAL CLOCK.

L. E. Fung, W. Li1, D. Setchachan, J. D. Miller1, P. Patel1, R. J. Dickerson2.

1Pharmacology, Texas Tech Health Science Center, Lubbock, TX and 2Cell and Neurobiology, Keck School of Medicine of USC, Los Angeles, CA.

Sleep disturbances and other neurological disorders are associated with occupational exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD, or dioxin). These problems may persist for years after exposure, significantly affecting quality of life. We hypothesize that TCDD causes persistent circadian rhythmic disturbances, which can be modeled in rodents. Using an infrared beam detection system, we monitored mouse locomotor activity continuously in the dark for several weeks. We found that TCDD doses as low as 10 ng/kg (gavage, in corn oil) induced persistent phase-shifts of intrinsic locomotor rhythm. However, the timing of the dose and the concentration were critical. The magnitude of the phase-shift was greater when the dose was administered by gavage (in corn oil) during the subjective night. Doses in the ng/kg range or less were associated with hyperactivity, while doses in the ug/kg range were associated with more severe pathological and biochemical changes, including weight loss, disturbed appearance, paw edema, and death after several weeks to months. Interestingly, this syndrome resembles rodent models of chronic sleep deprivation, as described in the scientific literature. Using immunohistochemistry and Western blot techniques, we characterized critical protein expression patterns within the suprachiasmatic nucleus (SCN) of the hypothalamus, where the master circadian pacemaker resides in mammals. Maximal diurnal expression of the clock-associated protein, BNMT-1, was delayed by hours by TCDD exposure during the light/dark cycle. Light and the endogenous pacemaker normally regulate the transcription of c-fos, an immediate-early gene. However, with TCDD, expression of c-fos protein was significantly increased in the SCN throughout the light/dark cycle. Entrainment of circadian rhythms to environmental chemicals represents a mechanism by which organisms may lose synchrony with their normal environment.

1634 EFFECTS OF TCDD ON RAT MOLAR DEVELOPMENT: THE CRITICAL TIME WINDOW.

H. Kattainen1, J. Tjuomisto2 and M. Viikela3. 1Department of Environmental Health, National Public Health Institute, Kuopio, Finland and 2University of Kuopio, Kuopio, Finland.

Dental defects, especially disturbances in tooth development, are sensitive end-points of dioxin-induced toxicity both in humans and animals. In rats TCDD exposure results in dental defects ranging from pulpal perforation in incisors to complete block of third molar development. We determined the critical time window for the effect of TCDD on rat molar development: in a TCDD-sensitive rat line. Dams were exposed to a single oral dose of 1 mg/kg TCDD on gestation day 1 (GD) 11, 13, or 15, or on postnatal day (PND) 2, 4, or 8. In a cross-fostering study dams were exposed on GD15 and the pups from each exposed dam were transferred to a non-exposed dam and vice versa immediately after delivery. Pups were killed at the age of 40 days, the eruption status of the third molar observed by stereomicroscopy, and the presence of the molars confirmed by radiography. Highest frequency of missing molars was observed in pups exposed both in utero and post-lactation, and only the development of third molars was blocked. Frequency of missing molars was the higher the earlier the dam was exposed: 64%, 50% and 26% of third molars were missing in offspring of dams exposed on GD11, 13 and 19, respectively. In the cross-fostering study, 13% of third molars were missing in pups exposed only in utero and no more than 1% of molars were missing in pups exposed only post-lactation. TCDD exposure time-dependently retarded the eruption of third molars, whereas the eruption of incisors and opening of the eyes was accelerated. The results indicate that the critical time window of molar development comes to the end right after parturition. However, continued exposure during lactation may further amplify the response. It is possible that TCDD affects incisors and molars by different mechanisms. Supported by the Academy of Finland, the Finnish Research Program on Environmental Health, Project 42551, and EC Contract QLK4-CT-1999-01446.

1635 SUSCEPTIBILITY LOCI FOR PORPHYRIA AND LIVER INJURY IN THE INTERACTION OF TCDD WITH IRON IN MICE.


Among the actions of the 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is the induction of hepatic porphyria. This is similar to the most common disease of this type in humans, sporadic porphyria cutanea tarda (PCT). Evidence is consistent with the action of TCDD being mediated through binding to the aryl hydrocarbon receptor with different Ah allele in mouse strains apparently accounting for differential enzymatic downregulation and susceptibility. However, studies of TCDD-induced porphyria and liver injury evidence indicate that there must be interactions with other susceptibility genes, perhaps associated with iron metabolism. We performed a quantitative trait locus (QTL) analysis of an F2 cross (204 individuals) between susceptible C57BL/6J (Ah+ allele) and the highly resistant DBA/2J (Ah- allele) strains after treatment with TCDD and iron. Genome-wide scans of crosses were performed using polymerase chain reaction amplification of microsatellite DNA markers. To determine the location of susceptibility genes, the extreme phenotype cohorts for hepatic porphyria, plasma ALT and AST together with thymus size (46 each) were initially genotyped for 44 markers spread across the genome at an average of 20 CM. After assessment by principle component analysis, all mice were genotyped with 6-9 markers on candidate chromosomess. For porphyria we found QTLs on chromosomes 11 and 14 in addition to
the Ab gene (chromosome 12). SWR mice are syngenic for the Abar allele with the DBA/2 strain but are susceptible to periphrasia after elevation of hepatic iron. Analysis of SWR x DBA/2 F2 mice (242) treated with iron and TCDD showed a QTL on chromosome 11, as well as finding others on chromosomes 1 and 9, for both periphrasia and plasma ALT as a measure of liver injury. These findings show, for the first time, the location of genes other than Ab that modulate the hepatic periphrasia and injury caused by TCDD in mice. Orthologous loci may contribute to the pathogenesis of human sporadic PCT.

1636
POSTNATAL DEVELOPMENT OF RESISTANCE TO ACUTE LETHALITY OF TCDD IN TWO TCDD-RESISTANT RAT STRAINS.
Department of Environmental Health, National Public Health Institute, Kuopio, Finland and National Food and Veterinary Research Institute, Helsinki, Finland.

The exceptionality resistance to acute lethality of 2, 3, 7, 8-tetrachlorodibenzop-p-dioxin (TCDD) is a prominent feature of TCDD-induced gene expression in the transactivation domain of the Ah receptor (AhR) in adult Han/Wistar (Kuopio) (H/W) rats. However, during fertility period H/W rats seem to be sensitive to at least teratogenic effects of TCDD. This study investigated the time window where resistance develops. H/W rats, line A rats (a new line with the H/W-type mutated AhR), and line B rats (line with a normal AhR but moderately resistant due to an unidentified gene) were exposed to a single i.p. dose 2 - 56 days after birth. At 21 days per group, 12 ml of TCDD (5 mg/kg); H/W and line A rats received 1000 µg/kg; male and female rats received 200 and 100 µg/kg, respectively. The LD50 doses of these strains/lives are 50000, >10000, 850, and 410 µg/kg, respectively. H/W rats were sensitive (25 - 65 % died within 42 days) if the dose was given on postnatal days (PND) 5 - 9, but were resistant thereafter (0 - 33 % mortality). Line A rats were sensitive on PND 2 (67 - 57 % mortality) but resistant after PND 5 (0 - 6 % mortality). Line B rats were very sensitive on PND 5 - 14 (all died) but developed resistance before PND 28 (no rats died if exposed after PND 28). The results show that H/W rats are sensitive to TCDD immediately after birth but develop the exceptional TCDD resistance to acute lethality within the first two weeks after birth. The moderate resistance of line B rats develops approximately at the time of weaning. It seems likely that within this time window there is a change in one or more AhR-mediated toxic pathways. Studies on this temporal change might bring forth new understanding on the mechanisms of action of AhR.

1637
AH+/− AND CYP1A2+/− KNOCKOUT MICE ARE PROTECTED FROM TCDD-STIMULATED INCREASES IN REACTIVE OXYGEN FROM LIVER MITOCHONDRIA.
Center for Environmental Genetics and Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

TCDD (2, 3, 7, 8-tetrachlorodibenzop-p-dioxin) causes oxidative stress (OS) in liver and extrahepatic tissues. We have previously shown that TCDD increases mitochondrial respiration-dependent reactive oxygen (RO) production, an important component of TCDD-induced OS. We herein examined the dependence of mitochondrial RO on the aromatic hydrocarbon receptor (AhR) or cytochrome P450 1A2 (CYP1A2), proteome critical for the activation of TCDD-induced hepatotoxicity. Congenic female AhR+/−, AhR−/−, Cyp1a2+/−, and Cyp1a2−/− mice were treated with TCDD (15 µg/kg, i.p.) or corn oil vehicle on 3 consecutive days; liver mitochondria were examined 1 week following the first treatment. Whereas elevated succinate-stimulated mitochondrial H2O2 production in the AhR+/− and Cyp1a2−/− mice, this increase did not occur in mitochondria from AhR−/− or Cyp1a2+/− mice. Interestingly, while constitutive levels of H2O2 production from the AhR+/− mitochondria were only 25% of that observed in AhR−/− mitochondria, there was no difference between Cyp1a2+/− and Cyp1a2−/− mitochondria.

Mitochondrial aconitase, an enzyme inactivated by superoxide, was decreased 44% by TCDD in the AhR+/− and Cyp1a2−/− mice but not in the AhR−/− or Cyp1a2+/− mice. Treatment increased mitochondrial glutathione levels in the AhR+/−, Cyp1a2+/− and Cyp1a2−/− mice, but not in the AhR−/− mice. These results suggest that the AhR controls the expression of genes involved in the TCDD-mediated mitochondrial RO production and that one of these genes is Cyp1a2. These results may be related to our observation, using Western immunoblot analysis, that TCDD elevates the accumulation of CYP1A1 and CYP1A2 proteins in liver mitochondria. The mechanism by which TCDD induces members cause mitochondrial OS may be of fundamental importance to TCDD-induced toxicity. Supported by NIH grants P01 ES06095, R01 ES01033, ES08147, and T32 ES07250.

1638
TCDD-INDUCED GENE EXPRESSION PROFILE SUGGESTS THE INVOLVEMENT OF DEATH-RECEPTOR PATHWAY LEADING TO INDUCTION OF APOPTOSIS IN THE THYMUS.
Microbiology and Immunology, MCV/VCU, Richmond, VA and Pharmacology and Toxicology, MCV/VCU, Richmond, VA.

Previous studies from our laboratory demonstrated that TCDD triggers apoptosis in thymocytes. Recently, two major apoptotic pathways in mammalian cells have been described. In the first pathway known as the death-receptor pathway, members including Fas (CD95) and TNF receptor 1 are activated. The second, mitochondrial pathway is triggered primarily through the activation of pro-apoptotic members of the Bcl-2 family. In the current study, we used cDNA arrays to detect the apoptotic pathway initiated by TCDD. To this end, C57BL/6 mice were injected with 0.1-0.5 µg/kg body weight of TCDD and 1 or 3 days later, thymus and liver were analyzed for a wide range of apoptotic genes. In the thymus, 33 out of 37 genes involved in apoptosis were up-regulated by TCDD by a factor of 2 or more. In contrast, in the liver, only 16 out of 37 apoptotic genes were up-regulated. In the thymus, several genes belonging to the death-receptor pathway were up-regulated including Fasl, caspase-8, caspase, CRADD, RIP, DAXX, TNF, TRAIL, TrID and TRIP following TCDD treatment. In contrast, the expression of anti-apoptotic members of Bcl-2 family including Bcl-xL and Bcl-2 and proapoptotic members such as Bad and Bax were not significantly up-regulated. In the spleen, 20 out of 22 apoptotic genes screened were up-regulated following TCDD treatment. The induction of caspases was seen at all doses of TCDD tested. Together, the current study suggests that TCDD treatment induces the death-receptor rather than the mitochondrial pathway in the sensitive organs to induce apoptosis (Supported in part by grants from NIH: ES09098 and HL38641).

1639
ROLE OF AH RECEPTOR IN TCDD-INDUCED APOPTOSIS IN THE THYMUS.
L. N. Canacho, M. Nagarkatti and P. S. Nagarkatti, Virginia Commonwealth University, Richmond, VA.

It is well established that 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent suppressor of the immune system. Exposure to TCDD has been associated with marked thymic atrophy. Despite extensive studies, the mechanistic aspects of TCDD immunotoxicity in the thymus are unclear. Many reports suggest that the immunotoxic effects of TCDD are regulated by the Aryl hydrocarbon receptor (AhR). Previous studies from our laboratory have demonstrated that TCDD induces apoptosis in thymocytes. In the current study, we tested the hypothesis that TCDD-induced apoptosis in thymocytes is regulated by the AhR. To test this hypothesis, alterations in the expression of various surface molecules in the thymus were measured as a predictive biomarker of apoptosis, as previously reported. To this end, C57BL/6 (Ah-high responder) and DBA/2 (Ah-low responder) mice were injected with 5 µg/kg TCDD (i.p.). Five days later, thymocytes were analyzed for phenotypic alterations using flow cytometry. At this dose, thymic atrophy was observed in TCDD-treated C57BL/6 but not DBA/2 mice when compared to the control mice. Consistent with our earlier reports, thymocytes from TCDD-treated C57BL/6 mice showed an increase in the density of expression of CD3 and CD44, and a decrease in the density of expression of CD4, CD8 and CD11d markers, when compared to the vehicle-treated control group. These phenotypic alterations were associated with thymocytes undergoing apoptosis. Interestingly, thymocytes from TCDD-treated DBA/2 mice did not exhibit any phenotypic changes characteristic of apoptotic thymocytes. The thymocytes of C57BL/6 wild-type mice when examined using pathway-specific cDNA microarray showed marked up-regulation in several genes involved in apoptosis. In contrast, AhR knockout mice showed no significant up-regulation in apoptotic genes. Together, these data suggest that TCDD triggers apoptosis in the thymus by an AhR-dependent mechanism. (Supported in part by grants from NIH: ES09098, F31ES11562-01 and HL38641).

1640
COMPARATIVE GENE EXPRESSION RESPONSES IN RATS FOLLOWING ACUTE AND CHRONIC EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).
Environmental Toxicology Program, NIEHS, Research Triangle Park, NC and NIEHS Microarray Center, NIEHS, Research Triangle Park, NC.

While humans are exposed to both persistent and non-persistent aromatic hydrocarbon receptor (AhR) ligands, it is believed that the sustained activation of the AhR response pathway by poorly metabolized ligands such as dioxins may be a key event in the development of associated adverse health effects such as cancer. The present study compares the in vivo response following acute or chronic exposure to
the mixed exposure group as compared to controls. Brain nuclear extracts from F1 pups were analyzed by EMSA on PND 0 through PND 30. Premature Sp1-DNA binding was observed on PND 5 in the mixed exposure group as compared to the control group. The results suggest that mixed TCCD/B(a)P exposure causes 1) modulation of the developmental expression profile of transcription factors involved in growth and differentiation in the developing CNS. A probable consequence of this modulation is to dysregulate the timetable of cellular development and growth in the CNS such that the phenotypic expression is 2) a significant reduction in spatial learning and memory. (Supported by U50NS41071, G12 R00 3032, ES 00287, and GM 000307 to DBH).

1643 MIDGESTATIONAL SUBACUTE, MIXED TCCD/B(a)P EXPOSURE: EFFECTS ON F1 GENERATION LONG-TERM POTENTIATION AND TEMPORAL EXPRESSION OF DEVELOPMENTAL CNS NEONATAL MARKERS I.

D. B. Hood, J. Wu, T. Nayyar, D. Wornimie, M. Greenwood and S. Chirwa, Pharmacology and Toxicology, Meharry Medical College, Nashville, TN.

The purpose of this study was to access the effects of mixed TCCD/B(a)P exposure to timed pregnant dams on the cellular basis of learning and memory in F1 generation offspring. Lapatroxy on GD 8 was performed on four groups of timed-pregnant rats including unexposed control, corn oil-diolein, B(a)P carbon black aerosol, and diolein-B(a)P carbon black aerosol. A dose of 700 ng/kg of diolein was given by gavage on GD 14 to timed-pregnant dams followed by dosing via nose-only exposure for four hours a day for four days (GD 14 through 17) to 0.1 mg/m3 B(a)P: carbon black aerosol. A significant (p<0.05) reduction in the birth index was observed in the diolein-B(a)P carbon black aerosol exposed group. There were no significant differences relative to controls in liver and/or brain to body weight ratios. Electrophysiological studies in the dentate gyrus revealed a significant diminution in I/O curves and in long-term potentiation in the mixed exposure group relative to controls. RI-PCR developmental expression profiles for AHR as well as western blot analyses from PND 0 through PND 30 revealed downregulation of AHR in the mixed exposure group compared to controls. Brain nuclear extracts from F1 pups were analyzed by EMSA on PND 0 through PND 30. Premature Sp1-DNA binding was observed on PND 5 in the mixed exposure group as compared to the control group. In conclusion, the mixed TCCD/B(a)P exposure was shown to 1) modulate the developmental expression profile of transcription factors involved in growth and differentiation in the developing CNS and 2) cause a significant reduction in a persistent synaptic enhancement induced by high frequency stimulation of afferents which has been suggested to be an important component of the cellular basis of forms of learning and memory. (Supported by U50NS41071, G12 R003032, ES 00287, and GM 000307 to DBH).

1644 MODULATION OF SELECTED APOPTOSIS-RELATED GENES DURING RETINOIC ACID-INDUCED DIFFERENTIATION IN MURINE EMBRYONIC STEM CELLS.

S. A. Sarkar and B. P. Sharma, Physiology and Pharmacology, The University of Georgia, Athens, GA.

Apoptosis plays an important role during embryonic development. Apoptotic cell death is executed by caspases and can be regulated by Bel-2 family genes. Selected apoptosis-related genes of the Bel-2 family, pro-apoptotic Bax and Bad, anti-apoptotic Bel-2 were investigated by ribonuclease protection assay (RPA) during differentiation of murine embryonic stem cells (ES), mediated by all-trans retinoic acid (RA). The mRNA expression of caspase 3, caspase 6 and certain proinflammatory cytokines were also investigated simultaneously. The ES cells mimicked in vivo differentiation and provide an excellent alternative method to study differentiation and apoptosis. The ES cells were grown on mitotically inactivated ST-166 fibroblast feeder layers in Dulbecco's modified Eagle's medium supplemented with retinoic acid. 15% Knock-Out Serum Replacement, leukemia inhibitory factor (LIF) and B-mercaptoethanol. Differentiation was initiated in the absence of feeder layers and LIF. Ribonuclease analysis revealed increased expression of Bax, Bad and no effect on Bel-2 mRNA in RA-treated cells compared to the vehicle-treated cells. Increased sense mRNA expression of caspase 3 and caspase 6 in RA-exposed ES cells suggested that caspases played an important role in RA-mediated apoptosis during ES differentiation. Increased expression of TNFα and macrophage migration inhibitory factor (MIF) was noted in RA-treated cells on day 14 as compared to vehicle-treated cells. Increased expression of interluekin gamma inducing factor (IL-18) RA-treated cells on day 14 and 17 did not translate to increased IFNγ expression. Expression of other proinflammatory cytokines was detected at only at background levels. Results suggested that RA-mediated apoptosis during differentiation of ES cells involves caspase 3, caspase 6, Bad and Bax. Our data is consistent with the biochemical process of apoptosis since it is relative lack of other proinflammatory cytokines except TNFα, IL-18 and MIF in RA-treated cells, whose function during ES differentiation is speculative.
MATERNAL IMMUNE STIMULATION REDUCES URETHANE-INDUCED CLEFT PALATE AND ALTERS MATERNAL SPLENIC AND FETAL PALATE GENE EXPRESSION.


Maternal immune stimulation by diverse techniques, prior to conception or during gestation, has been shown to be effective in reducing chemical-induced birth defects in mice. Morphologic lesions that are reduced include cleft palate and associated craniofacial defects, digit and limb defects, tail malformations, and neural tube defect, caused by chemical agents TCDD, urethane, methylnitrosourea, cyclophosphamide, or valproic acid. Limited information is available regarding mechanisms by which such immune stimulation reduced fetal dysmorphogenesis. In the present studies, immune stimulation in urethane-treated pregnant mice increased splenic leukocyte number, including T helper, B cells, and macrophages. An array consisting of 151 genes was used to study gene expression in leukocytes that control cell cycle and apoptosis, cytokine/interleukin production, and signal transduction and transcription regulatory factors important in development. As might be expected, maternal immune stimulation up-regulated expression of leukocyte genes involved in cell proliferation and cytokine production. Interestingly, expression of genes in the fetal palate, including bcl2, b, paka, and p53 was restored to control levels by maternal immune stimulation. Possible relationships between maternal immune stimulation and altered maternal and fetal gene expression, and reduced urethane-induced cleft palate, remain to be investigated.

EFFECTS OF PHORBOL 12-MYRISTATE 13-ACETATE (PMA) TREATMENT ON MORPHOLOGY AND GENE EXPRESSION OF XENOPUS EMBRYOS.

M. T. Murray, D. J. Kaplan, L. J. Suy, R. E. Novak, and H. Kim. Detroit Re STD., Detroit, MI and 'NIEHS Center, Wayne State University, Detroit, MI.

Effects of PMA treatment on morphology and gene expression were studied using embryos of Xenopus laevis at stages 8 (blastsula) and 15 (neural). Xenopus embryos were obtained by induction of ovulation followed by in vitro fertilization. Embryos at stage 8 were obtained after incubation of the fertilized embryos at 23°C for 5 hours. PMA (100 ng/ml) or DMSO (0.01%), solvent used to dissolve PMA was added to the embryos 2 hr after fertilization. Whereas embryos without PMA treatment progressed to blastula stage, PMA-treated embryos remained in a pre-blastula stage, suggesting that PMA treatment impaired differentiation. Embryos at stage 15 were obtained after incubation of the fertilized embryos at 18°C for 30 hours, PMA or DMSO was added to the embryos 21 hr after fertilization. Whereas embryos without PMA treatment showed typical morphology of neurula, PMA-treated embryos were in a pre-neural stage. Embryos treated with 4-alpha-PMA, a stereoisomer of PMA that is ineffective at activating protein kinase C, showed typical neural morphology. Embryos at stages 8 and 15 treated with PMA or DMSO were harvested and mRNA was isolated. The mRNA (35-50) was reverse-transcribed with oligo(dT) primer and dNTPs mixed with Cys-dUTP (DMSO control) or Cys-4-dUTP (PMA-treated). The Cys-3- and Cys-5-incorporated probes were mixed and microarray analyses were carried out using a glass chip printed with PCR products of Xenopus expression sequence tag (EST) clones (duplicate of 41, 100 genes).

Microarray analyses were carried out for stages 8 and 15 embryos treated and not treated. PMA-altered the gene expression profile differentially in the two embryonic stages. These results suggested, that whereas cell differentiation was blocked after treatment of Xenopus embryos with PMA at both pre-blastula and pre-neural stages, the PMA-responsive gene expression profiles were developmentally stage-specific. Sponsored by the NIEHS SBIR contract N43-ES-15462 & the NIEHS Center grant P30 ES06639.

TEMPORAL CHANGES IN THE EXPRESSION OF TRANSCRIPTION FACTORS IN THE DEVELOPING LEAD-EXPOSED HIPPOCAMPUS AS DETERMINED BY MACROARRAY ANALYSIS.

S. A. Bakheer and N. H. Zawia. Biomedical Sciences, URI, Kingston, RI.

The exposure to lead (Pb) may alter developmental gene expression and brain development through selective effects on the transcriptional machinery. In this study macroarray analysis was used to identify transcription factors that are involved in the response to Pb-exposure. Rat pups were exposed to Pb-acetate (0.2% from birth to weaning via lactating dams). On several postnatal days the hippocampi of control and lead-exposed pups were examined by macroarray analysis for changes in the development profiles of approximately 30 transcription factors, belonging to known transcription factor families. A number of transcription factors appear to be altered following lead exposure and have distinctive temporal patterns of expression. The most prominent changes were exhibited by the Sp and Oct families. Expression of Sp1 and Sp2 peaked on PND 5 and was diminished by PND 15 in Pb-exposed animals. By contrast, elevated Oct-1 & Oct-2 expression was sustained in these animals during the same period. These two transcription families appear to play a critical role in mediating Pb-induced disturbances in developmental gene expression. While the involvement of Sp transcription factors in the response to Pb exposure has been previously shown, the changes in Oct-1 and Oct-2 expression identify a novel target for Pb. Future work will focus on determining changes in Oct-1 and Oct-2 protein levels and DNA-binding and the role of the Oct family of transcription factors in the neurotoxic effects of Pb.

2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) REDUCES CARDIAC HYPOXIA AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN THE CHICK EMBRYO.

A. L. Sanchez, L. D. Aweeka and M. K. Walker. College of Pharmacy, University of New Mexico, Albuquerque, NM.

We have shown that TCDD inhibits coronary angiogenesis in the chick embryo. During normal embryo development cardiac hypoxia stimulates the expression of VEGF in the heart, which then stimulates coronary angiogenesis to occur. We tested the hypothesis that TCDD inhibits hypoxia-stimulated VEGF mRNA expression in the chick embryo heart. To estimate levels of cardiac hypoxia, we used a hypoxia-sensing drug, EFS, that is metabolically reduced by an oxygen-inhibitable microsorboase. When tissues are hypoxic, more EFS is reduced to a reactive intermediate which then binds to cellular macromolecules. Bound EFS was visualized using a Cy-3-conjugated antibody. VEGF mRNA expression was assessed by in situ hybridization. To confirm EFS binding and VEGF mRNA expression correlated with cardiac hypoxia, chick embryos on day 9 were incubated for 24 hr at 20% O2 (normoxic) or 15% O2 (hypoxic). Hearts from the hypoxic embryos showed increased EFS binding and VEGF mRNA expression, which localized primarily to the trabeculae, compared to hearts from normoxic embryos. To test the effect of TCDD on cardiac hypoxia and VEGF expression, fertile chicken eggs were incubated with 5 ml corn oil (control) or 0.3 pmol TCDD/ml in 5 ml corn oil prior to incubation. On day 10 semiquantitative analysis of EFS staining showed a significantly decreased level of binding of EFS at normoxic levels. Qualitative analysis showed that TCDD treatment decreased VEGF mRNA expression in the cardiac trabeculae as compared to controls. We conclude that TCDD may inhibit coronary angiogenesis by down-regulating hypoxia-induced VEGF mRNA expression. Supported by NIH grant ES-09804.

ANALYSIS OF GENE EXPRESSION PROFILES IN DIFFERENTIATING MURINE EMBRYONIC STEM CELLS.


Pluripotent embryonic stem (ES) cells of the mouse are able to differentiate in vitro into a variety of different cell types. In the embryonic stem cell test (EST), an in vivo assay for the determination of the teratogenic potential of compounds, ES cells of the mouse (cell-line D3) are investigated for their preserved potential to differentiate into contracting cardiomyocytes following compound exposure. The inhibitory effect of compound on differentiation is compared with its cytotoxicity on ES cells and on differentiated mouse 3T3 fibroblasts. To improve the EST, molecular endpoints of differentiation were examined for their potential to predict the embryotoxic potential of a compound. Gene expression of the ES cells was measured at different time points after initiation of differentiation using oligonucleotide microarrays (Affymetrix). The effect of the strongly embryotoxic compounds 5-fluorouracil and all-trans retinoic acid, the weakly embryotoxic diphénylhydantoin and dexamethasone as well as the non embryotoxic perindol and aciclovir were investigated in comparison with untreated controls. Among the genes showing the strongest up-regulation in untreated cells between day 7 and day 10 of differentiation compared to undifferentiated ES cells, several genes coding for cardiac specific proteins could be found. This included atrial/fetal and ventricular myosin light chain 1, myosin light chain 2, alpha and beta cardiac myosin heavy chain and cardiac troponins. Also, up-regulation of cell-specific genes like hematopoietic, endothelial or neural genes could be seen. 5-fluorouracil and all-trans retinoic acid as well as diphénylhydantoin led to a down-regulation of the relative expression of cardiac specific genes, while dexamethasone induced an up-regulation of cardiac genes. Penicilllin and aciclovir did not significantly affect the relative expression of cardiac genes. In conclusion, gene expression represents a suitable endpoint to study compound induced changes in cardiac differentiation. The implementation in the EST will be evaluated.
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DEVELOPMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS EXERTS THYROID HORMONE-LIKE EFFECTS ON THE EXPRESSION OF THYROID HORMONE-RESPONSIVE GENES IN THE FETAL RAT BRAIN.

K. J. Gauger, C. T. Herzog and R. T. Zoeller, Biology, University of Massachusetts, Amherst, MA.

Background exposure to polychlorinated biphenyls (PCBs) during fetal development is associated with lower IQ and attention deficit in humans and clear neural defects in experimental animals. These effects may be caused by interfering with thyroid hormone signaling in much as PCBs reduce serum thyroid hormone. However, the structure of some individual PCB congeners is reminiscent of that of thyroid hormone and some authors propose that PCBs may interact directly with the thyroid hormone receptor. Therefore, we initiated a study to determine whether maternal exposure to PCBs has an effect on specific thyroid hormone-responsive genes in the fetal rat brain, including RC3/Neurogranin, Neuronodocrine-Specific Protein A (NSP-A), and Oct-1. NSP-C, which is not affected by maternal thyroid status, was used as a negative control. Using in situ hybridization, we have found that PCBs increase the expression of RC3/Neurogranin and do not affect NSP-C expression.

The remaining analyses are forthcoming. These studies are the first to address the effect of PCBs on thyroid hormone action in the fetal rat brain, an important question considering that all humans are contaminated with measurable levels of PCBs. Moreover, they justify a concerted effort to test whether individual PCB congeners can bind to the thyroid hormone receptor and transactivate gene expression. Supported by ES10026.

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EFFECTS OF SINGLE COMPONENTS (HYPERTHERMIA & BORIC ACID) AND MIXTURES ON MID-ORGANOGENESIS STAGE-RAT EMBRYOS USING MICROARRAY ANALYSIS.

W. A. Harrouk1, M. B. Rosen, S. E. Petron1, J. R. E. Schmidt, G. L. Kimmel2 and C. A. Kimmel1, DLIS/CDHR, USFDA, Rockville, MD; 1NHEERL, RTD, DBR, USEPA, Research Triangle Park, NC; 2NCEA, ORD, USEPA, Washington, DC.

The goal of this project is to examine the interaction of two developmentally toxicants, hyperthermia and boric acid (BA), and their effects on the gene expression profile of mid-organogenesis rat embryos following maternal in vivo exposure. When administered singly on gestational day (GD) 10 in the rat, both agents cause similar developmental toxicity profiles (malformed/reduced numbers of thoracic vertebrae & ribs). When used in combination, the interaction of BA (oral dosing, 500 mg/kg) and hyperthermia (41°C & 42°C, rectal temp maintained for 5 min) was synergistic as manifested by an increased prevalence of neural, thoracic, lumbar and sacral defects, and reduced numbers of presacral vertebrae. These effects seem to target newly developed somites or the presomatic mesoderm at the time of exposure. We have shown that adverse morphological, biochemical and molecular alterations occur as early as 3-h post exposure. Here, we have used the microarray approach to map the gene expression profile in embryonic tissues containing the somitic region isolated 5 hrs after in vivo exposure to BA, 41°C or 42°C. Briefly, 33P labeled cDNA was synthesized from total embryonic RNA by reverse transcription. Labeled cDNAs were then hybridized to Atlas Rat Toxicology 1.2 arrayTM and exposures were recorded using an FX phosphorimager. The relative expression of each gene was determined using Atlas ImageTM software. Preliminary normalized data focusing on sets of genes belonging to DNA damage & repair, apoptosis, heat shock and cell signaling pathways indicate upregulated expression following 42°C and even greater with BA+ 42°C exposure but not 41°C or BA+ 41°C, suggestive of a synergetic mechanism of action in the high dose combination. Long term efforts are to build predictive models for risk assessment for single and combined exposures. Supported by a fellowship to WAH by ORISE. This abstract does not necessarily reflect EPA or FDA policies.

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MATERNAL THYROID HORMONE INCREASES THE EXPRESSION OF GENES THAT AFFECT GLIAL DETERMINATION IN THE EARLY ERYPT.

R. Bansal, C. A. Herzog, E. A. Iannaccone and R. T. Zoeller, Biology, University of Massachusetts, Amherst, MA.

Thyroid hormone (TH) is well known to play essential roles in brain development in both humans and animals. However, the specific developmental events affected by thyroid hormone are poorly understood. As a result, risk assessment is not based on specific points of thyroid hormone action, often making the assignment of adverse effects difficult. To identify specific end points of TH action in the fetal brain, we have begun to examine members of the Nodch signaling pathway, which transduces cell-cell contact information on decisions of cell fate during cortical development. The target genes of the pathway are the basic Helix-Loop-Helix (bHLH) genes Hairy Enhancer of Split homologues 1 and 5 (HES-1, HES-5), which encode transcriptional repressors thought to antagonize protomeric bHLH genes and favor glioneogenesis in the cortex. To test whether TH of maternal origin affects Nodch signaling in the fetus, we treated pregnant rats (n=35) in 6 of 9 ways: 1) Untreated 2) propylthiouracil (PTU) 3) Salmon 3) PTU 4) one of two doses of T4 5) one of two doses of T4 6) one of two doses of T4 7) one of two doses of T4 8) one of two doses of T4 9) one of two doses of T4. Animals were sacrificed on gestational day 16 when the fetal cortex is actively producing both neurons and glia. Semi-quantitative in situ hybridization revealed that PTU decreased HES1 expression and T4 restored or increased HES 1 expression in a specific region within the fetal cortex. We conclude that thyroid hormone affects the Nodch signaling pathway in the fetal cortex. Indeed, we have found that TH does not affect neuronal proliferation in the early cortex; therefore, we are pursuing the hypothesis that TH increases the formation of glia at the expense of neurogenesis. Funded in part by ES10026.

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INTERSPECIES DIFFERENCES IN BBDR MODEL INPUT PARAMETERS AND THEIR IMPLICATION FOR MODELING TUMOR INCIDENCE.

I. A. Lewandowski1, C. S. Wells2 and R. L. Rhoenberger, 1Graduate Corporation, Mercer Island, WA and 2Graduate Corporation, Cambridge, MA.

The utility of biologically-based Dose-Response (BBDR) models for predicting cancer risks in humans could be increased if we understood how model parameters scale across species and what these scaling relationships indicate about species-specific cancer risks. Yet parameter selection is often treated as trivial relative to statistical aspects of model formulation. We are identifying species-specific values from the literature for the initial cell number and rates of cell birth, death and mutation in both normal and initiated cells. Such data are difficult to obtain because they are rarely the focus of published studies or abstracts and must be gleaned from control data. Collection of cell cycle kinetic and cell number data for several tissues of interest has revealed varied patterns in cross-species scaling. For example, while hepatocyte number increases in a ratio of 1:3-850 among mice, rats and humans, thyroid follicular cell number increases in a ratio of 1:2.04500. Hepatocyte number scales linearly with organ weight but follicular cell number deviates from linearity due tostructural differences in the rodent and human thyroid. Background levels of cell proliferation and apoptosis are similar among the three species, with greater differencesbetween tissues than between species. A review of spontaneous mutation frequencies indicates that, across all three species, mutation frequencies for all tissues except lymphocytes generally range from 1 x 10^-6 to 8 x 10^-5 per cell. Thus, most of the key parameters in a Moolgavkar-type carcinogenesis model appear to be similar across species. We are now developing a modeling approach in which these similarities in input parameters, species life cycle differences, and the background tumor incidence rates in rodent and human populations can be considered in a coherent manner. Other factors, such as different rates of DNA repair may also be included in the modeling. This work is supported by funding from the American Chemistry Council.

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APPLYING THE B6C3F1 MICE GROWTH MODEL USING WEIGHT DATA FROM A NTP BIOASSAY STUDY: ASSESSING DOSE-RESPONSE EFFECTS OF p-NITROBENZOIC ACID.

J. T. Walker1 and O. A. Walker1, 1National Center for Environmental Assessment, USEPA, Washington, DC and 2TJW Research Institute, Upper Marlboro, MD. Sponsor: D. Singh.

Biologically based Dose-Response (BBDR) models are being used increasingly by regulatory agencies for predicting biological risks in terms of the dose level of a particular chemical. The primary purpose of the dose-response models is to provide a descriptive summary of experimental data as function of dose. For BBDR models that are based on growth, empirical models establishing relationships between body weights and age in growing animals are needed. In this paper, we applied our newly developed B6C3F1 mice growth model in assessing the dose-response effects of p-nitrobenzoic acid on the growth curves and parameters of mice that were involved in a NTP bioassay study. Individual growth curves of these model is provide a nonlinear least squares technique. Our main objective was to determine if the chemical caused dose-dependent changes in the mean values of the model parameters. Standard linear regression techniques were used to determine if the parameter
values varied with dose. The results showed that most of the parameters demonstrated a significant dose-response trend; some increased with dose, while others decreased. Some showed no significant changes, however. These results are preliminary, but important, for three primary reasons. First, they show evidence which suggests that low chronic levels of p-nitrobenzoic acid cause dose-dependent changes in the growth parameters of living mice. Second, although the model is purely empirical, it is a potentially useful tool for analyzing B6C3F1 mice weight growth data from NTP bioassay studies. Third, because the parameters of the model represent biological indices of growth, they can be used for BBIDR modeling purposes. Disclaimer: The views expressed in this paper are those of the author(s) and do not necessarily reflect the views or policies of the USEPA.

1655 PROBABILISTIC FRAMEWORK FOR NON-CANCER RISK ASSESSMENTS.

M. Pelekos, M. J. Nicolich and J. Gaethier. Toxicology & Environmental Sciences Division, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ.

Non-cancer human health risk assessments use point values to develop risk estimates and thus impart a deterministic character to risk, which by definition is a probability phenomenon. The objective of the present work was to develop a probabilistic framework that eliminates the deficiencies of point values and estimates non-cancer risk across the population without the use of uncertainty factors. Probability distribution functions (PDFs) were incorporated within a physiological modeling framework to account for the propagation of uncertainty/variability in exposure, kinetics and dynamics of volatile organic compounds. The integrated physiological-based toxicokinetic-toxicodynamic model for the dichloromethane (DCM)-induced carboxyhemoglobin in humans was used to illustrate the proposed probabilistic framework. The mechanistic parameters were represented by (1) PDFs which provided both the value of the parameters and their frequency of occurrence and (2) the correlations among the parameters to characterize the inter-relationships among the parameters. Ambient exposure concentration was linked to dose absorbed by PDFs that described the variability of DCM exposure indoors and outdoors, exposure frequency, exposure duration (exposure time) and population characteristics such as gender, age, and inhalation rates at different activity levels. The median and 95% confidence interval of carboxyhemoglobin (HBCO) in blood after a lifetime of continuous exposure were estimated. The distribution of the risk (i.e., HBCO in the population) was thereby determined by taking into account the combined effects of the multiple sources of uncertainty/variability encountered in the exposure-tissue dose-tissue response continuum without the use of uncertainty factors. This approach provides an alternative to the default methodology. It shows that application of probability into non-cancer risk assessments can reduce uncertainties when establishing exposure limits for specific compounds and provide better assurance that established limits are adequately protective.

1656 A BIOLOGICALLY-BASED DYNAMIC MODEL FOR PREDICTING THE DISPOSITION OF FORMALDEHYDE AND ITS METABOLITES IN ANIMALS.

M. Bouchard, R. C. Brunet, P. O. Droz and G. Carrier. Environmental and Occupational Health, Chair of Toxicological Risk Assessment for Human Health, Université de Montréal, Montreal, PQ, Canada.

A multi-compartment biologically-based dynamic model was developed to describe the time evolution of formaldehyde biodistribution in animals (rats, mice and rabbits) following different formaldehyde exposure scenarios. A model previously developed to describe the biodistribution of methanol and its metabolites in animals and humans (Bouchard et al., 2001) was used as a framework for the development of this model since methanol is metabolized mainly to formaldehyde in the animal and human body. In the model, a certain fraction of formaldehyde is readily oxidized to formate, a major fraction of which is rapidly converted to CO2 and exhaled. The rest of formaldehyde is converted to unobserved by-products which are represented by a compartment with slow elimination. The dynamic of inter-compartment exchanges was described mathematically by a mass balance differential equation system. Parameters were estimated from metabolism time profiles published in the literature following either methanol or formaldehyde exposure. Data were available over longer periods of time after exposure to formaldehyde than was the case following methanol exposure. These data showed that time profiles of CO2 exhalation in rats, mice and rabbits followed bi-exponential functions. The formaldehyde exposure was not apparent from previously published data on methanol exposure. The model reproduces these patterns by introducing a slow elimination rate of unobserved formaldehyde by-products through metabolism to CO2 and exhalation, in addition to the more rapid elimination from the formate body burden.

1657 REACTION NETWORK MODELING: A NEW MODELING APPROACH FOR BENZO(A)PYRENE BIOLOGICAL PATHWAYS.

K. H. Liao, W. Wei, M. T. Klein, K. F. Reardon and R. S. Yang, "Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Colorado State University, Fort Collins, CO, 2Department of Chemical Engineering, Colorado State University, Fort Collins, CO and 3Department of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey, Piscataway, NJ.

A new modeling approach, BioMOL (Biological application of Molecular Oriented Lumping), has been used to simulate the metabolic pathways of xenobiotics in a biological system. By setting the reaction rules, the reaction network is generated automatically at the molecular level. Graph theory is used to convert chemical structures and reaction rules into computer code. The atomic connectivity of the molecules is represented by the bond-electron matrix. The reaction matrix is a compact representation of bond formation and breakage caused by a chemical reaction. BENZO(A)PYRENE was used to build the first model by BioMOL since it is a human carcinogen as well as an ubiquitous environmental pollutant. Its metabolic pathway has been extensively studied and a great deal of information is available in the biomedical literature. The reaction rules used in this model include epoxidation of double bonds, hydrolysis of epoxide, NIH shift of arene oxide to phenol, oxidation of phenol to quinone, glucuronidation, sulfation, and glutathione conjugation. The rate constants are estimated based on the reaction family concept and Quantitative Structure Reactivity Correlations (QSRCs). The reactivity index, which is related to the reaction rate constants by QSRCs, can be calculated by BioMOL-integrated semi-empirical quantum chemistry software. Candidate reactivity index include heat of reaction, electron density, and bond order. Key parameters were determined in a normal human epidermal keratinocyte cell culture system and used to verify the model built by BioMOL. This approach will be a promising tool for complex biological processes involved in chemical and chemical mixture toxicity. This work was supported by NIHES Grant # R01 ES09655.
previously published studies. Detoxification pathways included enzymatic pathways mediated by cytochrome P450 and A C-esterase, as well as non-enzymatic pathways involving butyrylcholinesterase and carboxylesterase. The pharmacodynamic model describing the interaction of parathion with acetylcholinesterase included phosphorylation of the active site, as well as a peripheral binding site, that was not occupied when the active site was occupied by parathion. The apparent Ks (app) for parathion when the peripheral site was unoccupied, determined experimentally, was 0.90 PM h\(^{-1}\), while the same constant when the peripheral site was occupied by parathion was 0.000000125 PM h\(^{-1}\). Other significant events included the pharmacodynamic model were reactivation of inhibited enzyme, aging, and normal synthesis and degradation of enzyme. Comparison of the simple model's predictions with data from various publications involving different routes of administration of parathion and parathion in the rat, indicated that the model accurately simulated the pharmacokinetic disposition of parathion and parathion, and accurately simulated the degree of acetylcholinesterase inhibition in brain, diaphragm and blood, as well as butyrylcholinesterase and carboxylesterase inhibition in liver, blood, and heart tissue following exposure to parathion or parathion.

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BIOLLOGICALLY BASED MODELING OF CHLOROFORM CYTOLETHALITY AND REGENERATIVE CELLULAR PROLIFERATION.

Y. Tan, B.E. Butterworth and R.B. Caudy,
Center for Computational Biology and Genomic Modeling, CIT Center for Health Research, Research Triangle Park, NC and Bateson Consulting, Raleigh, NC.

The cytotoxicity of chloroform occurs when the rate of generation of toxic metabolites exceeds the capacity of cellular protective mechanisms. In this study, a PBPK model for chloroform was extended to include cytolethality and regenerative cellular proliferation. This allowed calculation of a simulated labeling index (LI) that was compared with LI data from chloroform-exposed B6C3F1 mice and F344 rats. Key challenges in developing the model were (1) identifying the optimal form of the function linking chloroform metabolism to cytolethality and (2) defining a mechanism controlling the rate and extent of RCP. Possible forms of the linking function include (a) the rate of metabolism, (b) the AUC for the rate of metabolism over some interval, (c) the rate of metabolism at a minimum rate, and (d) the AUC for the rate of metabolism at a minimum rate over some interval. The actual biological mechanism of RCP is not fully understood, but involves growth factors and signaling pathways. In the interest of model parsimony, a simplified signaling submodel was developed. In this submodel, liver cells secrete a signal into blood and normal liver size is associated with a specific blood level of a signal. As cytotoxicity reduces the number of cells, the level of signal falls. The difference between the control and exposed levels of signal is used as a driving force to increase the rate of cell division. This is an efficient means of simulating the LI data. Simulations with rates of metabolism and the AUC for rate of metabolism showing submodels did not accurately describe the LI data, and the more complex submodels were being evaluated. This work will increase our understanding of the quantitative relationship between chloroform pharmacokinetics and cytolethality and support biologically based modeling of the dose-response for chloroform-induced cytolethality in humans.

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A COMPUTER DOCKING STUDY OF THE BINDING OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR METABOLITES TO THE LIGAND BINDING DOMAIN OF THE ESTROGEN RECEPTOR.


Polycyclic aromatic hydrocarbons (PAH) are a class of ubiquitous, anthropogenic chemicals found in the environment. In the present study, we use computational methods to evaluate their potential estrogenicity and the contribution of PAH metabolites in this class make to environmental estrogenicity. Classical docking methods, Dock and AutoDock, are used to evaluate the potential binding affinity of chemicals in this class and their metabolites to the published crystal structures of the ligand binding domain of the estrogen receptor. These methods, with a molecular mechanics interaction energy scoring function, were able to place estradiol within an RMSD of 3 Å from its binding position determined by x-ray crystallography. The scores obtained in this manner show wide variation for the PAHs and their metabolites. They depend on PAH type and the three dimensional structure of the metabolites. For example, (1-anti-benz[b]chrysenephenanthrene diol epoxide is much better binder in this model than the (1-enantiomer. A few dominant modes of binding have been identified and will be presented. These results will be compared to the results for known binders. Semi-empirical quantum mechanical methods were also used to compute the interaction energy of the most stable structures obtained from the classical computer docking experiments. These quantum mechanical calculations provide a quantitative description of the interaction between the ligand and the receptor and contain elements that are not obtained from the classical scoring function. The comparison of these results demonstrates the importance of the nonclassical interaction terms for molecules that have pi electron systems. (This work does not necessarily reflect EPA policy. K.W. Brown was funded by EPA/UNNC Toxicology Research Program Training Agreement CT9709298 and CT827266.)

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THE EFFECTS OF ATRAZINE ON THE ABILITY OF ESTRADIOL TO BIND TO ESTROGEN RECEPTOR IN THE HYPOthalamus.

T. S. McMullen, L. A. Nagahara, R. J. Handa and M. L. Andreen, Environmental Health, Colorado State University, Fort Collins, CO and Anatomy and Neurobiology, Colorado State University, Fort Collins, CO.

Atrazine (ATRA), a chloro-triazine herbicide, is widely applied throughout the United States. At high doses, it suppresses the prolactin (LH) surge in female rats. Although the site of action of the hypothalamus, the mechanism is unknown. Estradiol (E2) acts on hypothalamic neurons to initiate the LH surge. Chloro-triazines have weak anti-estrogenic effects in some assays. Our studies determined if ATRAs anti-estrogenic properties are responsible for the diminished LH surge in female rats. We verified earlier binding studies with ATRA and E2. ATRA (750 µM) caused a dose-dependent inhibition of E2 binding to ER, independent of pre-incubation. Decreased responsiveness of the hypothalamus to E2, caused by ATRA, could interfere with the E2 - mediated positive feedback required for the LH surge. To test this, the following in vitro studies were performed: (1) we confirmed the ability of ATRA to blunt the LH surge in ovarectomized, E2 treated female rats. At 300 mg/kg/day ATRA significantly suppressed the surge. The remaining studies used this treatment regimen. (2) Using in-situ hybridization, we assessed changes in progesterone receptor (PR) mRNA expression following in vivo exposure to ATRA. PR mRNA is upregulated in response to activation of ER and also when E2 is added to E2 receptor deficient U2-OS cells, in dopamine neurons. If ATRA decreased ability to bind ER, PR mRNA would not be increased. ATRA treatment caused no change in PR mRNA expression in various hypothalamic regions compared to controls. (3) In vivo F1K nuclear exchange assays determined changes of occupied hypothalamic ER after ATRA and estrogen treatments. ATRA had no effect on the ability of ER to bind E2. Therefore, despite evidence of anti-estrogenicity, this property of ATRA is unlikely to be involved in suppression of the LH surge. This research was funded by Superfund Grant (5 P42 ES05949).

1662A

A SHORT-TERM DOSING MODEL FOR DETECTING THE EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON THYROID HORMONES IN THE RATS EFFECTS OF PESTICIDES.

J. M. Hedge, D. G. Ross, M.L. DeVito and K.M. Carton, Neurotoxicology Division, USEPA, Research Triangle Park, NC and Environmental Toxicology Division, USEPA, Research Triangle Park, NC.

Recently, a short-term rat dosing model has been developed to examine the effects of environmental mixtures on thyroid homeostasis (TH). Prototypic chemicals such as diulosins, polychlorinated biphenyls and polynitrophenyl diethyl ethers have been tested and shown to adversely impact both neurological development and TH, primarily by up regulating calcium and thyroid hormones by liver enzymes. Current efforts examined the effects of select pesticides in this model. Female Long Evans rats (28 days old) were orally dosed for four consecutive days with the dihydrobenzeno furicidic acid methanes (MAN) or the herbicide promamide (PRO) (0, 3.9, 7.8, 5.6, 32.5, 62.5, 125, 250, 500, 1000, 10,000 mg/kg/day), or the dihydrobenzeno furicidic acid synthesis (TH) (10, 25, 50, 100, 200, 300, 400, 800 mg/kg/day). Serum and liver samples were collected 24 hrs after the last dose. Triiodothyronine (T3) and thyroxine (T4) were measured via radioimmunoassays. Hepatic ethoxyresoruf-O-deethylase (EROD), pentoxifylline-O-deethylase (PROD), and arylhydrocarbon-hydroxylase (AHH) were determined in hepatic microsomal fractions. Liver-to-body weight ratios (LBR) increased 3 days, at doses >25 mg/kg/day. MAN at >800 mg/kg/day, and TH at >400 mg/kg/day. PRO, MAN and TH all produced similar dose-related decreases in T4, with estimated ED50's of approximately 250 mg/kg/day. Potencies for decreases in T3 were TH > PRO > MAN. Maximal suppression of T3 and 14 was >50% and 80% at the highest doses, respectively. Neither MAN or TH caused changes in EROD or PROD. These data suggest that this rodent dosing model is sensitive to short-term perturbations in thyroid hormones caused by these pesticides. This abstract does not necessarily reflect the policy of the USEPA.
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**17γ-ESTRADIOL AND 4-HYDROXYTAMOXIFEN-INDUCED TRANSLATION ACTIVATION IN BREAST AND ENDOMETRIAL CANCER CELLS IS DEPENDENT ON ER SUBTYPE, CELL AND PROMOTER CONTEXT.**

E. Castro-Rivera and S. H. Safe. Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.

The pattern of transcriptional activation by 17γ-estradiol (E2) and 4-hydroxytamoxifen (4-OHT) was determined in ZR-75 and MDA-MB-231 breast, ECCI and HECA1 endometrial and HePG2 liver cancer cells cotransfected with E2-responsive constructs and wild-type estrogen receptor α (ER-α) or ERβ (ER-β) or variant forms of ERα influencing transcription function (ER-α F1; ER-α F2) or ERβ (ER-β F2). The E2-responsive constructs contained promoter inserts from the human complement C3 (pC3-luc), human cathespin D (pCDAT) and rat creatine kinase B (pCKB-CAT) genes. Minimal ERβ-dependent transcription (2.5-fold induction) was observed for E2 only in ECCI and MDA-MB-231 cells transfected with pCDAT or pC3-luc, whereas 4-OHT was inactive as an ERγ agonist for all promoters in the four cell lines. The pattern of E2- or 4-OHT-activated gene expression and their interactions were highly complex and dependent on cell type and promoter context and form of cotransfected ERα. For example, E2 did not activate pC3 in HePG2 cells transfected with wild-type or variant ERα, whereas E2 activated reporter gene activity in the four endometrial/breast cancer cell lines transfected with ER-α WT and pCDAT or pC3,5 but not pC3. However, hormone activation of these constructs by ER-α F1 or ER-α F2 was highly variable among the different cell lines and even in the same cell line transfected with the three E2-responsive constructs. Similar variability was observed for 4-OHT suggesting that transcriptional activation by E2 and 4-OHT induces recruitment of different transcription factor complexes that are dependent not only on the cell type but also the gene promoter. (Supported by NIH ES04917 and ES09106)

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**EVALUATION OF POTENTIAL ESTROGENIC PROPERTIES OF OCTAMETHYLCYCLOTETRAISOXANE (D4) USING THE MCF-7 CELL LINE.**


The objective of this research was to evaluate the potential estrogenic properties of octamethylcyclotetrasiloxane (D4). Using an oral estrogen responsive reporter gene system followed by Northern blot analysis, D4, estradiol (E2), and diethylstilbestrol were evaluated using the MCF-7 human cell line. Treatment with D4 for 24 hours at an applied dose of 0.1 - 10 µM resulted in a dose-dependent increase in p52 expression. As expected, all of the positive control compounds showed significant increase in mRNA expression. Linear regression analyses indicated that D4 was 74, 009 times less potent than E2 with the compounds showing equivalent efficacy at the highest dose. Maximal mRNA expression was seen between 6 and 24 hours, although exposure at shorter times resulted in increasing levels of mRNA. The loss of D4 in the medium was monitored and there was a rapid loss of compound, which reached a plateau around 12 hours with less than 10% remaining. The potential for D4 to act as an anti-estrogen was evaluated comparing the E2 Dose-Response curve with and without co-injection of 1.0 µM D4. This regression analysis resulted in parallel slopes with statistically indistinguishable y-intercepts, indicating that the D4 is not reducing the potency of E2 under these conditions. Preliminary receptor binding studies were carried out to examine the binding of D4 to ER-α and ER-β. Studies indicate that the D4 can weakly compete for the estrogen receptors, both ER-α and ER-β. D4 did not reduce E2 binding beyond 75% of the control value even at 0.1 µM concentration of 250 µM. This data suggests that D4 can elicit an estrogenic effect that is dose-dependent with no significant anti-estrogenic activity. Binding data indicate that the effect is mediated through the estrogen receptor. Supported in part by Silicones Environmental, Health and Safety Council of North America.

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**CELL CONTEXT-DEPENDENT DIFFERENCES IN THE MECHANISM OF ESTROGEN REGULATION OF E2F1 IN BREAST CANCER CELLS.**

S. Nwennyia and S. H. Safe. Biochemistry & Biophysics, Texas A&M University, College Station, TX and Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.

E2F1 is a member of the E2F family of transcription factors which plays an important role in cell cycle progression and proliferation. E2F1 is induced by 17γ-estradiol (E2) in breast cancer cells and contributes to hormone-induced proliferation of estrogen receptor (ER)-positive cell lines. Mutation and deletion analysis of the E2-responsive -146 to -54 or -169 to -54 regions of the E2F1 gene promoter indicates that hormone-induced transcription in MCF-7 cells requires at least one of three G-rich sites (-169 to -111) that bind ERα/Sp1 and cooperatively interact with two downstream CCAAT motifs (-122 to -54) that bind NFYA. Transient transfection of E2-responsive constructs pE2F1a (-169 to -54) or pE2F1b (-146 to -54) containing E2F1 gene promoter inserts are induced ≥3.5-fold in both ER-positive MCF-7 and ZR-75 cells. Gel mobility assays of nuclear extracts from both cell lines gave comparable patterns of retarded bands and confirm that Sp1 and NFYA proteins bind upstream (p169/111) and downstream (p122/54) CCAAT motifs, respectively. Gene expression analysis of the E2F1 promoter in ZR-75 cells using pE2F1c and pE2F1d (containing -169 to -111 and -122 to -54 E2F1 promoter inserts) showed that E2 induced a 2.5- and 3.5-fold increase in reporter gene activity, whereas these constructs were inactive in MCF-7 cells. These results demonstrate that E2F1 is differentially regulated by co-operative ERα/Sp1 and NFYA interactions in ZR-75 cells and by independent ERα/Sp1 and ERα activation of CCAAT binding proteins in ZR-75 cells. Current studies are investigating the novel genomic/nongenomic pathways associated with hormonal activation through CCAAT motifs. (Supported by NIH ES04917 and ES09106)

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**ESTROGENIC RESPONSES TO GENESTEIN IN JAPANESE MEDAKA (ORYZIAS LATIPES).**

L. Zhang, A. K. Khan and C. M. Foran. Department of Pharmacology, The University of Mississippi, University, MS; Department of Pharmacology, The University of Mississippi, University, MS and Department of Biology, West Virginia University, Morgantown, WV. Sponsor: K. Willer.

Although the phytoestrogen genistein has been determined to be weakly estrogenic, the change in endocrine function associated with genistein exposure is not necessarily expected to mirror the physiological changes following exposure to estrogenic estrogens. Because of the potential for genistein to compete with 17β-estradiol at the receptor, some estrogenic responses might be diminished in the presence of genistein. Thus, this study was designed to determine the estrogenic effect of the phytoestrogen genistein on endocrine physiology in adult Japanese medaka (Oryzias latipes) relative to 17β-estradiol. Adult animals of both sexes were exposed to 750 µg/fish, 750 ng/fish, and 30 µg/fish (average fish weight was 0.26 g/fish) of genistein by i.p. injection. A positive control group was similarly treated with 3000 ng/fish of 17β-estradiol, while a negative control group received a vehicle (corn oil) injection. Several assays were used to determine the extent and type of changes in the endocrine system of treated animals. Vitellogenin, while induced by estradiol, was not increased in the liver of individuals treated with genistein. Genistein treatment at higher doses increased the estradiol production by ovaries more than estradiol treatment. In males, genistein treatment resulted in decreased testosterone production from ex vivo tests and a comparable reduction in circulating testosterone level. The changes in endocrine physiology in medaka in response to genistein are similar to that of estradiol. However, some endpoints are more sensitive to estradiol treatment (vitellogenin) while others are more sensitive to genistein (male testостерон and female ovarian estrogenesis). Further studies with dietary supplements will build on these results, which will serve as a basis for testing individual components of plant extracts for estrogenic activity.

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**DIFFERENTIAL EXPRESSION OF THREE ESTROGEN RECEPTOR ISOTYPES DURING LARGE MOUTH BASS (MICROTERRORUS SALMONIDES) REPRODUCTION AND FOLLOWING EXPOSURE TO NONYLPHENOL.**

T. L. Sabo-Attwood, M. S. Nelson, J. L. Upp and N. D. Dentlou, Pharmacology and Therapeutics, Molecular Genetics and Microbiology, and Biochemistry and Molecular Biology, University of Florida, Gainesville, FL.

Discovery of multiple estrogen receptor (ER) isotypes raises questions regarding their role in gene regulation by endogenous estradiol and xenestrogens. Here we report the discovery of three ER isotypes in largemouth bass (LMB), ERα, ERβ, and ERγ. Using real-time quantitative PCR we have determined that these isotypes are differentially expressed in brain, pituitary, gonad, and liver tissues in vitellogenic females. For example, ERα and ERβ are more highly expressed than ERγ in the liver. However, ERγ expression is greater than both of the other ER isotypes in gonadal tissue. This differential tissue distribution pattern suggests they play very different roles during the reproductive process. To begin to understand their roles in reproduction we measured the expression pattern of each ER in adult female LMB during normal oocyte maturation. Liver tissue was collected at approximately two-week intervals from October through June. Expression of each ER and vitellogenin (VTG) mRNA were quantitated using real-time PCR. We also measured plasma VTG protein levels by direct ELISA. Results show that each ER isoform displays a distinct pattern of expression. There is an apparent correlation between ERα and VTG mRNA and VTG protein levels. ERγ however, appears to be down regulated during peak vitellogenesis. ER expression remains fairly constant throughout reproduction suggesting it plays a minor role in gene regulation in the liver. To
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THE ESTROGENIC PROPERTIES OF 3, 3'-DIINDOLYL METHANE (DIM) ARE DEPENDENT UPON CYTOCHROME P450 (CYP) METABOLISM

S.C. Thompson, A.D. Shilling and D.E. Williams 
Environmental and Molecular Toxicology and The Linus Pauling Institute, Oregon State University, Corvallis, OR and Department of Drug Safety and Metabolism, Wyeth-Ayerst Research, Collegville, PA.

Indole-3-carbinol (I3C) is a glucosinolate hydrolysate product from cruciferous vegetables that has been found to have chemoprotective effects in several animal models. However, I3C given as long-term postinjection promotes liver carcinogenesis in mouse and rat models, but inhibits hepatic tumors in mice similar to the actions of 17b-estradiol (E2). A major acid condensation product of I3C, DIM, is estrogenic in premenopausal women and accounts for the majority of the estrogenicity of I3C in vitro and in vivo. This evidence, along with the species specificity of I3C as a hepatic tumor modulator, suggests an estrogenic mechanism of action for hepatocarcinogenesis. In this study, we demonstrate that DIM requires CYP-dependent met- abolism to exert its active estrogen effects. The results were incubated with DMSO, 100 mM E2 or 20 uM DIM. CYP metabolism was inhibited in these slices with 20 uM SKF 525A (SKF) or ketoconazole (KC). Vitellogenin (VTG) induc- tion in E2-treated slices was not affected by either CYP inhibitor whereas VTG induc- tion by DIM was significantly decreased 79% and 80% in slices treated with KC and SKF, respectively (p<0.05). These data further support the importance of estrogenic mechanisms in tumor modulation by I3C and suggest that species differences in hepatic tumor promotion are due to the CYP-specific metabolism of DIM to estrogenic metabolites. Supported by PHG grants ES03850 and ES07060 from the NIEH.

1669

THE REGULATION OF THE STEROIDISOMIC ACUTE REGULATORY PROTEIN (STAR) PROTEIN IN LARGEBONE BASS (MICROPTERUS SALMOIDES).

Biochemistry, University of Florida, Gainesville, FL.

The Steroidogenic Acute Regulatory Protein (STAR) transports cholesterol across the mitochondria into the secretory granule, making cholesterol available to be metabolized into steroid hormones. Some environmental contaminants have been found to alter the levels of steroid hormones, which could occur through targeting the expression of the STAR Protein. We obtained gonad samples of largemouth bass (LMB) at two week intervals throughout a year to determine the normal mRNA levels of the STAR Protein and its correlation with steroid hormone levels. Total RNA was isolated from gonads and mRNA levels of STAR were then quantitated using real-time PCR with Taqman technology. Primers for the real-time PCR assay were designed from a partial, 345 base pair sequence of the LMB STAR Protein previously cloned in our Lab. The levels of STAR in LMB go from an average of 0.2 pg STAR/mRNA/ug of total RNA during the quiescent period in the fall to a peak of 7.3 pg STAR/mRNA/ug of RNA during the primary reproductive season. The expression pattern of STAR correlates with the expression pattern of 17-beta-estradiol, as would be expected since it is the rate limiting step in steroid synthesis. Establishing the normal levels of STAR in LMB during the reproductive season will set baseline parameters for studying the effects of environmental contaminants on STAR expression. It has been shown already that several contaminants, including herbicides and insecticides, decrease the expression of the STAR Protein in mammalian cell lines (Walsh et al, 2000, Environ Health Perspectives, 108(8):769). Fish exposed to contami- nants from paper mills have been extensively studied. A new class of fish, the deerhead chub, has been identified as a good indicator of ionic strength levels, possibly through alteration of STAR expression. LMB is an excellent model for studying the effects of contaminants on the regulation of the STAR Protein.

1670

4-NONYPHENOL ALTERS STEROID HORMONE METABOLISM IN THE LIVER OF FVB MICE.

R. Acvedo, R. Robinson, S. Cox and W.S. Baldwin
Biological Sciences, University of Texas at El Paso, El Paso, TX and Biology, Anderson College, Anderson, SC.

4-Nonylphenol (NP) is an endocrine disrupting chemical that enhances proliferation of MCF-7 breast cancer cells due to its affinity for the estrogen receptor. Recent data also indicates that NP binds to the PXR receptor (Massuyama et al., 2000), therefore it has the potential to alter CYP 3A family member expression. These P450s are important in the inactivation and 6beta-hydroxylation of testosterone. Because PXR receptor activation appears to alter xenobiotic and steroid me- tabolism, we hypothesized that NP may alter steroid hydroxylation and possibly steroid conjugation. This may be important in activating 4-NP’s own estrogen or the elimination of other chemicals; however, it is also possible that 4-NP may alter steroid hormone metabolism in ways that are not beneficial to the organism. Female FVB mice were treated for seven days with 0, 25, 50, and 75mg/kg/day 4- NP in honey, and then euthanized. Livers were excised, microsomes and cytosol prepared and then both estradiol and testosterone glucuronidation, sulfation and hydroxylation was examined. 4-NP significantly increased estradiol sulfotransferase- activity 59% at 75mg/kg/day 4-NP and in a dose-dependent fashion, but 4-NP did not increase estradiol or testosterone glucuronotransferase activity. 4-NP did signifi- cantly inhibit both estradiol and testosterone glucuronotransferase activity in vitro. We are currently investigating 4-NP’s actions on estradiol and testosterone hydroxylation and conjugation, and those results will be presented. These alterations in steroid conjugation may effect clearance of steroids from exposed tissues, and thus increase the potential of 4-NP to promote steroidocarcinogenicity.

1671

STIMULATION OF THE THYROID GLAND WITH THYROTROPIN RELEASING HORMONE IN THE RAT: VALIDATION OF THE MEASUREMENT OF THYROID STIMULATING HORMONE.

Toxicology and Pathology, Limburg Research Foundation, Beersel, Belgium. Sponsor: W. Powiers.

The purpose of this study was to validate the measurement of serum Thyroid Stimulating Hormone (TSH) with a rat specific radioimmunoassay (Amersham, UK) after stimulation with commercial Thyrotropin Releasing Hormone (TRH). In a group of male and female rats, TSH levels were measured at 15, 30 and 60 minutes after a single IV injection of 100 mg/kg body weight. Serum TSH levels were measured by a competitive protein binding assay. Serum TSH was measured at 15, 30 and 60 minutes after stimulation with 100 mg/kg body weight IV. Serum TSH levels did not increase significantly. The mean level of TSH increased from 4.4 mIU/L to 4.6 mIU/L at 15 minutes and decreased slightly at 30 and 60 minutes. The observed values were within the normal range for rats. The assay was shown to be sensitive and specific for measuring serum TSH levels. The assay was valid for use in the rat.

1672

PERCHLORATE INDUCED THYROID HORMONAL CHANGES IN PREGNANT RATS AND FETUSES.

Gn-e-cen-sas, Inc, Weight Patterson Air Force Base, O H and "AFRL/HEST, Weight Patterson Air Force Base, O H and "The University of Georgia, Athens, GA.

The production and storage of ammonium perchlorate (AP) has resulted in the contamination of soil, ground and drinking water in a number of states. The per- chlorate ion interferes with iodide uptake in the thyroid, resulting in reduced thy- roidal hormone synthesis. In rodents, this leads to lower triiodothyronine (T3) and thyroxine (T4) levels in the blood, and the activation of thyroid stimulating hormone (TSH). A time-course kinetic study with perchlorate was conducted to ex-amine perchlorate kinetics and corresponding hormone changes in pregnant rats. Pregnant rats (n=8) were administered perchlorate at a concentration of either 0 or 0.1 mg/kg via tail vein on gestation day 20 (GD20) and sacrificed at 1, 4, 8, 12, 24 and 48h post dosing. Maternally derived fetal serum perchlorate concentrations, determined at all time points, reached their maximum at 4 hours after perchlorate expo- sure. Serum perchlorate concentrations were undetectable at 24 and 48h post dosing in the dams, as well as in the fetuses they were undetectable at 8, 12, 24 and 48h post dosing. Serum levels of TSH, T3 and T4 were measured in both the dams and fetuses. In the 0.1-mg/kg-dose group serum TSH levels were increased significantly (p<0.05) at 4h and 8h post dosing in both the dams and fetuses. Serum thyroid
hormones decreased significantly (p<0.01) at 4th post dosing in the 0.1 mg/kg per platelet dose group in both the dam and frusine. Maternal and fetal serum TSH levels at 0.1 mg/kg per platelet dose in the 8th post dosing groups were 5.47 ± 0.34 and 6.06 ± 0.45 ng/mL as compared to maternal and fetal serum TSH levels of 4.00 ± 0.38 and 5.11 ± 0.49 ng/mL in the control groups at GD20. Fetal Serum TSH levels were significantly decreased at 4th, 8th and 12h post dosing in the 0.1 mg/kg dose group. Assessment of thyroid, pituitary, and adrenal using the most sensitive end point to understand platelet effects during gestation and provide data to develop a pharmacodynamic model for platelet in rodents.

1673 EXPRESSION PROFILE IN LIVER, PITUITARY, AND THYROID FROM PHENOBARBITAL TREATED RATS.

Phenobarbital is a microsomal enzyme inducer that causes thyroid follicular cell hyperplasia and tumors in rats, presumably by increasing the clearance of thyroid hormone and stimulating hormone. Increased clearance of T3 and T4 is due in part to induction of UDP-glucuronosyl transferase (UGT). The induction by phenobarbital in the liver is well documented, however its effects in the thyroid and pituitary have not been reported. Through the use of DNA arrays, the effect of phenobarbital (100 mg/kg/day) on the expression of 8,740 genes in the liver, thyroid, and pituitary of male rats treated for 14 days was measured. Several xenobiotic metabolizing enzymes, including CYP2B and 3A family, as well as UGTs were induced in all three tissues. Specifically, CYP3A1 was induced 3.4, 15, and 14.4-fold, CYP3A3 was induced 2.6, 11.9, and 18.7-fold, CYP2B2 was induced 52.1, 67.2, and 21.4, CYP2B19 was induced 9.8, 27, and 37.3-fold, and UGT2B2 was induced 4.4, 4.5, and 19.5-fold in rat liver, pituitary, and adrenal, respectively. These findings suggest that thyroid hormones metabolize in extrahepatic tissues could contribute to the mechanism of phenobarbital induced tumors in the rat by reducing hormone levels at the site of hormone regulation.

1674 SHORT-TERM EXPOSURE TO AN ENVIRONMENTAL MIXTURE OF PFAs: DOSE-ADDITIVE EFFECTS ON SERUM THYROXINE.
K. M. Custer1, R. W. Setzer1, J. M. Hege1, D. G. Rozell1 and M. L. De Villeg1
1Neurotoxicology Division MD-74B, USEPA, Research Triangle Park, NC and 2Experimental Toxicology Division, USEPA, Research Triangle Park, NC.

Endocrine disruption from environmental contaminants has been linked to a broad spectrum of adverse outcomes. An additional concern about endocrine disrupting xenobiotics is the potential for additive or synergistic effects of mixtures. A short-term dosing model to examine the effects of environmental mixtures on thyroid hormone homeostasis has been developed. Porcine chemicals such as dioxins, polychlorinated biphenyls, and polyfluorinated hexafluorobenzenes have been shown to alter thyroid hormone homeostasis in this model primarily by up regulating hepatic catabolism of thyroid hormones. Current efforts are testing the hypothesis that the effects of mixtures of these chemicals on thyroid hormone concentrations can be predicted by dose-additivity theory. The current study used 28 day-old female Long-Evans rats, orally dosed with varying concentrations of 18 different PFASs: 12 dioxins, 4 dibenzo-furan, and 12 PCBs, including dioxin-like and non-dioxin-like PCBs for four consecutive days. Serum samples were collected 24 hours after the last dose. Serum total T4 was measured via radioimmunoassay. Extensive (7-9 dose/chemical) dose-response functions were statistically modeled to determine ED70s using the Hill equation. A mixture was custom synthesized with the ratio of chemicals based on environmental concentrations (e.g., relative to TCCD: TCDF: TCEP: 1.5x, PCB126:90x, PCB153:30x, 000x). Serial dilutions of this mixture were tested in the 4-day dosing assay. Predicted outcomes based on the assumption of dose additivity were tested using statistical dose-response modeling. Preliminary analysis of results suggest that the effects of the mixture on serum total T4 can be predicted by dose additivity. There was no evidence of synergism or antagonism. Future work will expand the mixture to include chemicals from diverse classes of thyroid disruptors such as TH synthesis inhibitors. This abstract does not necessarily reflect the policy of the USEPA.

1675 DDE PARTIALLY RESTORES ANDROGEN-PRESSED TRANSCRIPTATIONAL ACTIVITY OF THE CONSTITUTIVELY ACTIVE RECEPTOR (CAR).
M. E. Wyde1, A. Ueda1, M. Negishi1 and L. You1. 1CIT Centers for Health Research, Research Triangle Park, NC and 2NIEHS, Research Triangle Park, NC.

The constitutively active receptor (CAR) is an orphan nuclear receptor that primarily regulates the transcriptional induction of cytochrome P450 (CYP)2B but also cross-regulates the CYP 3A gene. Steroid hormones are CAR ligands and affect CAR-mediated regulation of CYP 3A and 2B expression. CYP 3A and 2B are involved in the metabolism of steroid hormones. Xenobiotics have also been demonstrated to activate or repress CAR-mediated transcriptional regulation of enzyme expression. Alterations in CAR-regulated CYP 3A and 2B expression may lead to enhanced steroid catabolism and alterations in endocrine function. The aim of this current study was to test the hypothesis that the antiandrogen 1,2-dichloro-2,2 bis[p-chlorophenyl]ethylphenoxy (p,p-DDE) alters CAR-dependent transcriptional activity. A schematic showing CAR response to chemical exposure was determined in cultured HeptG2 cells that were co-transfected with mouse CAR expression plasmid, a control vector (pRL-SV40), and the luciferase reporter gene construct containing the CAR nuclear receptor binding site NR1. CAR activation was lower in cells treated with testosterone and other androgens than in cells treated with vehicle alone. No significant effect was observed in CAR activation following exposure to DDE alone at concentrations ranging from 0.01 to 50 μM. However, transcriptional activation of CAR was partially restored by DDE in cells treated with androgens. These data indicate that DDE mitigates androgen-induced repression of CAR transcriptional activation and suggest involvement of CAR in the antiandrogenic effects of DDE.

1676 INTERACTION OF TRENBOLONE, A POTENTIAL FEEDLOT EFFLUENT CONTAMINANT, WITH HUMAN AND RAINBOW TROUT ANDROGEN RECEPTORS (AR) IN VITRO AND IN VIVO ANDROGENIC EFFECTS IN THE RAT.
V. S. Wilson1, C. Lambright1, J. Ostby1, E. M. Wilson2, M. Cardon1, P. Harig1, L. J. Guillette1 and L. E. Gray1. 1RTD, USEPA, ORD, NHEERL, Research Triangle Park, NC and 2UNC, Chapel Hill, NC and 3UFL, Gainesville, FL.

Concern has arisen regarding the presence and persistence of trenbolone in the environment. Trenbolone acetate is an anabolic steroid used to promote growth in cattle. It is hydrolyzed to the active compound, 17 beta-trenbolone, which is one of the primary metabolites then excreted by cattle. Both reproductive alterations in fish living in waters receiving feedlot effluent and in vitro androgenic activity in feedlot effluent samples have been reported. In the current study, the potency of 17 beta-trenbolone was examined both in vitro and in an in vivo screening assay for androgenic activity. In COS cell binding assays using transfection of rainbow trout or human AR, trenbolone competed equally well for [3H]R1881 binding to either AR, with relative binding affinities of 4.9% and 3.4%, respectively, versus 100% for R1881. Trenbolone was also tested for its ability to activate androgen-dependent transcription at concentrations ranging from 1 μM to 10 μM in MDA-Mb247 cells with endogenous human AR and a stably transfected androgen responsive luciferase reporter. Trenbolone was a full AR agonist in these cells producing a Dose-Response curve similar to that of dihydrotestosterone. Furthermore, agonistic activity was inhibited by the AR antagonist, hydroxyflutamide. Trenbolone was also potent AR agonist in the NC terminal cell assay performed in HeLa cells, an assay that is highly specific for androgens. When examined in vivo in the Hershberger assay, trenbolone displayed selective AR mediated (SARM) activity, affecting some androgen-dependent tissues in rats more than others suggesting that 5 alpha reduction converted the parent compound. In summary, trenbolone is a potent SARM. Further studies will investigate the complete mechanism of feed-lot effluent and to characterize the ability of this chemical to alter vertebrate reproduction and development.

1677 POST-TRANSCRIPTIONAL EVENTS ARE INVOLVED IN CYPROHEPATADINE INDUCED INHIBITION OF PREPROINSULIN SYNTHESIS IN RINm5F CELLS.
B. S. Havigson and L. L. Fiechter. Department of Pharmacology and Toxicology and Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.

Previous studies have demonstrated that the antiinsitamic, anticonvulsant drug cyproheptadine (CPH) inhibits insulin synthesis in RINm5F cells without an alteration in preproinsulin mRNA (PpI mRNA) levels. This suggests that the compound is acting via one or more post-transcriptional mechanisms to inhibit the synthesis of insulin. A critical step in secretory protein synthesis is the translation of the ribosome-associated nascent chain from the cytosolic compartment, where protein synthesis begins, into the lumen of the endoplasmic reticulum (ER) where translation continues. Using a subcellular fractionation technique followed by real-time RT-PCR, the percentage of PpI mRNA in various stages of ribosome association was examined. Control cells showed 62% ± 2.0% of PpI mRNA in ER-bound form and 31.6 ± 3.4% in translationally-initiated free cytosolic form while a two hour treatment with 10μM CPH altered these distributions to 38.7 ± 3.9% and 52.9 ± 4.9% respectively. CPH also induced an increase in the percentage of translationally-terminated free PpI mRNA. This CPH induced dissolution of PpI mRNA from ER-bound form to free cytosolic forms was found to be concentration dependent, evident within thirty minutes, and reversible upon removal of the compound. Also,
the ability of CPH to induce PnMmRNA expression was found to be chemical structure specific as the CPH analog 2-diphynylmethyldiphenylmethylpyridine, active in inhibition of CTP synthetase, elicited similar PnMmRNA induction while the CPH analog 2-diphynylmethyldiphenylpyridine, inactive in inhibition of CTP synthetase, did not.

Overall, these data provide evidence supporting an effect of CPH on PnMmRNA translation. CPH may act to inhibit CTP synthetase by altering one or more of the processes involved in the initiation or elongation stages of translation or in the binding of ribosomes to the ER.

1678 AROMATASE INHIBITION BY AZOLE DRUGS: ASSESSING PREGNANCY RISKS.
S. D. Turner, 1 L. Kragie, T. He, 2 C. L. Cress, 1 and D. M. Strooper. 1 BD Bioscience, Wehurn, MA and EndoInc. Health Foundation Ltd, Silver Spring, MD.

Human aromatase (CYP19) converts C19 androgens to aromatized C19 estrogenic steroids that are important for pregnancy maintenance and also in regulating uterine contractions in late pregnancy. Inhibitors of this enzyme are associated with fetal loss in non-human primates. We tested a panel of azole antifungals which may be administered to pregnant women for their potential to inhibit aromatase using the fluorometric substrate dichromylfluorescein and cDNA-expressed CYP19. Potency varied by several orders of magnitude. For example, memantine was consistently inactive as an inhibitor at concentrations up to 100 μM while halofenate and miconazole returned IC50 values of 1.5 μM and 0.04 μM, respectively. Typical plasma levels of some azole drugs (e.g. miconazole) following oral or topical administration are at or above these IC50 values. These data suggest that some azole compounds may disrupt estrogen production in pregnancy and may affect pregnancy outcome.

1679 EFFECTS ON AROMATASE ACTIVITY IN MAMMALIAN CELL LINES AFTER EXPOSURE TO VARIOUS PESTICIDES.

Imidacloprid, carbendimid, epoxiconazole and atrazine are widely used pesticides in Europe and the USA. These compounds contain a triazine, imidazole or triazole ring and previous research suggests that these molecular structures are, at least partly, responsible for interaction with aromatase (CYP19). Interference with the aromatase enzyme can cause an alteration in steroidogenesis, leading to an enhanced or impaired conversion of androgens to estrogens. We used the rat Leydig tumor cell line R2C to assess the effects of aromatase activity after a 24h exposure to these pesticides. Concentrations ranged from 0.25 to 100 mM. Aromatase activity, with a V0 value of 25 mM and amoxicillin, was measured using a mini-H2O release assay. Corticosteroid effects of the pesticides were assessed by performing an MTX test for cell viability. Exposure of R2C cells to imidacloprid resulted in more than a 5 fold induction of aromatase activity at concentrations > 30 mM, while no effect on cell viability was observed up to 100 mM. Imidacloprid and atrazine showed an inhibitory effect on aromatase activity at concentrations > 3 mM and > 10 mM, respectively. Both pesticides caused a decrease in cell viability at concentrations > 100 mM. Atrazine did not affect aromatase activity nor cell viability in R2C cells, whereas it was shown to cause induction of aromatase activity in the human adrenocortical carcinoma cell line NCI-H295R. These results suggest different mechanisms of action concerning regulation of aromatase activity within the different cell systems. It is concluded that these commonly used pesticides have the potential to interfere with androgen metabolism and could possibly be endocrine disruptors in the in vivo situation. Further research on the effects of these pesticides and on their mechanism of action concerning regulation of aromatase activity within the NCI-H295R cell line and the rat Leydig carcinoma cell line 1C-540 is in progress.

1680 PUBERTAL DEVELOPMENT IN FEMALE WISTAR RATS FOLLOWING EXPOSURE TO PROPANIDE AND ATRAZINE METABOLITES, DIAMINO-S-CHLOROTHRIAZINE AND HYDROXYAATRAZINE.

We have shown that the chlorothriftazine herbicide, atrazine (ATR), delays the onset of pubertal development in female rats. ATR and its hydroxylated metabolites are present in soil and groundwater. Since current maximum contaminant levels are set only for ATR, it is important to determine whether or not these metabolites can alter pubertal development and possibly pose a cumulative exposure hazard. Here, we have evaluated the effects of two ATR metabolites, diamino-s-chlorothriftazine (DACT) and hydroxyaatrazine (OH-ATR), and a structurally similar chlorothriftazine, propazine (PRO), on female pubertal development. Rats were gavaged from postnatal day (PND) 22 through PND41 with DACT (16.5, 33.7, 67.5, 135 mg/kg), OH-ATR (22.8, 45.7, 91.4, 183 mg/kg), or PRO (26.5, 53, 107, 213 mg/kg). The dose range for each chemical was selected as the molar equivalent of ATR (25, 50, 100, 200 mg/kg). Females were weighed twice daily at 8:00 AM (before vaginal opening (VO) and killed on PND 41 for DACT and delayed VO by 3, 4, 5, and 7 days compared to controls (32.7 ± 1.8 days of age) after doses of 33.7, 67.5 and 135 mg/kg, respectively. The NOAEL for DACT (16.5 mg/kg) was identical to the equimolar NOAEL for ATR (25 mg/kg). Body weight (BW) on PND41 was reduced only by the high dose of DACT (7.1%; p<0.05). The percentage of rats with delayed VO was associated with delayed VO. Also, the BW on the day of VO was increased by 33.7, 67.5 and 135 mg/kg DACT. PRO (107 or 213 mg/kg) delayed VO by 4 days, but did not alter BW on PND41. In contrast, the dechlorinated metabolite, OH-ATR, did not significantly delay the age at VO, suggesting that the presence of the chlorine group may be necessary for activity. Together, these data demonstrate that both PRO and DACT can delay the onset of puberty in the female rat at doses equivalent to that of ATR, and suggest that environmental exposure to multiple chlorothriftazines and metabolites may pose a cumulative hazard. This abstract does not reflect USEPA policy.

1681 EFFECT OF AN ACUTE EXPOSURE TO MOLITANE ON OVULATION IN THE RAT.

Molinate, a thiocarbamate herbicide used on rice, has been shown to impair fertility in the male rat. In a two-generation study, molinate exposure to female rats resulted in decreased litter size and ovarian abnormalities. However, published data is lacking on the effects of acute exposure to molinate in the female rat. We reported complete inhibition of ovulation and our previous observations with related triazolamides, we hypothesized that a single exposure to molinate during the critical window for the neural trigger of ovulation on proestrus would alter the timing of ovulation. Female gavaged at 0, 25 or 50 mg/kg molinate on proestrus were examined for ovulation and ovary weight 24 or 48h later. All controls ovulated at 24h. Molinate at 25 mg/kg delayed ovulation for 24h in 43% of the rats, while 50 mg/kg delayed ovulation in 85%. The number of ovulations present in the delayed females was unaltered. To examine LH secretion, ovarecetomized (O VX) rats were implanted with physiological levels of estradiol (E2) and the induced LH secretion determined by tail bleed at 0, 2, 4 or 6h after a single oral dose at 1300h. 25 mg/kg molinate caused a significant suppression of the LH in 62% of the rats (4h mean = 0.566 ± 0.01 mg/ml vs 3.560 ± 0.7 mg/ml in controls). 50 mg/kg molinate suppressed the LH surge in 83.3% of the rats (4h mean = 0.347 ± 0.1 mg/ml). In another study, OVX/E2 rats were killed at 0, 1, 3 and 6h after similar dosing, 50 mg/kg suppressed the LH surge in 75% of the rats, while 25 mg/kg suppressed the LH surge in 63% of the rats. These results indicate that molinate is able to delay ovulation by suppressing the LH surge on the day of proestrus and that the brain and/or pituitary are target sites for this pesticide. These effects may explain in part the effects observed in previously published chronic studies with molinate in the female rat. Because delayed ovulation can result in altered pregnancy outcome and reduced litter size, it will be important to determine the precise CNS/Pituitary mechanism involved in this effect. This abstract does not reflect USEPA policy.

1682 EFFECTS OF OCTAMETHYLCYCLOTRISILANOLAE (D4) AND PHENOBARBITAL (PB) ON LH SURGE AND OVULATION IN SPRAGUE-DAWLEY (SD) RATS.

This study was conducted to assess the potential mechanism of previously observed reproductive effects of D4 in SD rats. Cannulated SD rats were exposed to 0, 700, or 900 ppm D4 vapor for 3 days (6 hrs/day) from estrus day-1 into the day of proestrus. A different set of rats received saline or PB (40 mg/kg, i.p.) on the day of proestrus at 1:45 p.m. Blood for the analysis of LH was collected with autologous replacement on the day of proestrus at 2, 4, 6, 8, and 10 p.m. Trunk blood was collected on the day of estrus at 8 a.m. for the evaluation of plasma estradiol (E2) levels in D4- and PB-treated rats. Ova were counted in wet mounts of fresh ovarics. The ovaries were routinely fixed and processed for light microscopy. D4 exposure increased the percentage of rats with attenuated LH surge at 4, 6, 8, and 8 p.m. in 900 ppm group, and at 6 p.m. in 700 ppm group relative to controls. Similarly, the LH surge in PB-treated rats was suppressed at all time points. In D4-treated rats, E2 levels on the day of estrus were higher both in 700 (22.2 ± 4 pg/ml) and 900 ppm (21.1 ± 1.8 pg/ml) groups relative to controls (7.8 ± 1.4 pg/ml). Plasma E2 levels in PB group were also elevated (21.2 ± 2.7 pg/ml vs. 8.7 ± 2.1 pg/ml in controls), but not statistically significant. D4 and PB both significantly decreased...
the percentage of rats that ovulated. Among ovulators, they had little effect on the number of ova produced. Rats that failed to ovulate had numerous mature follicles and lacked basophilic (new) corpora lutea as evidenced by histopathology. Control and treated rats that failed to ovulate had similar hormonal and ovarian morphology and only basal development, for retrieval of ovaries treated rats that ovulated normally were also similar. These results indicate that D4 induced PRL and PRL suppression effects on LH and ovulation. Supported in part by Siemens Environmental, Health, and Safety Council of North America.

1683 THE EFFECT OF MELATONIN AGAINST RADIATION INDUCED DAMAGE IN ALBINO RATS.

Melatonin is a hormone produced by the pineal gland and has been shown to have radioprotective effects. The objective of this study was to determine the potential radioprotective effects of melatonin. Adult rats were exposed to an oral dose of 250 mg/kg melatonin one hour before exposure to whole-body gamma radiation of a single dose 8 Gy (Lethal dose 1D50/30). The animals were observed for mortality over a period of 30 days following irradiation. A second group was treated with oral 1.02 and 250 mg/kg melatonin one hour before exposure to whole-body gamma radiation of single dose 4 Gy (lethal dose). Two days post exposure, animals were sacrificed and the following were analyzed: histopathology, lipid peroxidation, bone marrow, blood cytology and biochemical studies. A third group was treated oral doses of 250 mg/kg melatonin one hour before exposure to whole-body gamma radiation of multiple doses of 1 Gy per week for 6 weeks (Fractionated doses). Two days post exposure, surviving animals were sacrificed and the same analysis performed. The data obtained in this study suggest that melatonin at a dose as high as 250 mg/kg is non-toxic, and that high doses of melatonin are effective in protection of rats from effects of lethal, sublethal and fractionated doses of whole-body irradiation.

1684 PLACENTAL RESPONSES TO BUTADIENE DIOXIDE TREATMENT IN PREGNANT RATS.
E. Sponsler, E. Nixon and L. Chi, Biology, Southern University, Baton Rouge, LA.

Butadiene dioxide (BDE) is a metabolite of 1,3-butadiene that is reproducion toxic in rats and mice, and is utilized in synthetic rubber production. In rats specifically, since BDE-induced reductions in ovulation and fertility, the study was to clarify biochemical and physiological responses to implantation BDE exposure (gestation days 5-8). Gestation in female Sprague-Dawley rats 220-250g, maintained under 12:12 of h light-dark cycle, was initiated by cohabitation between a fertile male and proestrous female rats. Radiography (0.05-0.09g/kg body wt) were treated with 5.0 daily 0900-1000h intraperitoneal doses of BDE 0.05, 0.25, 0.30, 0.35 or 0.40 mmol/kg in saline. The dosing volume was 2.5 ml/kg. Animals were sacrificed on gestation day 9 or 12 0900-1000h, the period of placental and early fetal development of the rat. Tissue samples were analyzed for hepatic and placental samples by bromosulfophthalein (BSP) clearance and placental carryover levels using RIA. The results analyzed by Student's t-test, showed that in pregnant BDE-treated rats, the BSP clearance was reduced in placental samples, but not in serum levels. These reductions were significant when measured on either gestation day 9 or 12. Declines in implantation sites and corpora luteal levels were more consistent after gestation day 12 than at day 9. However, fetal mortality was drastically increased overall by 100% on gestation day 12 compared to a general increase of 55% on gestation day 9. The data suggest that the presumptive anti-placental biochemical and developmental actions of post-implantation BDE treatment in pregnant rats are apparently more critical as fetal development progressed from gestation day 9 to day 12. Supported by NIH/NSF Research Grant ES 10018-02

1685 INDUCTION OF VITELLOGENIN IN RESPONSE TO GENISTEIN FROM A SOY BASED DIET IN CHANNEL CATFISH.
E. C. Meek and J. E. Chambers, Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.

&$#77529$Genisteen, 4', 5', 7', 5'-tetrahydroxyisoflavone, is a phytoestrogen contained in soy. Soy is a major component of many commercial catfish feeds. Genisteen may induce the hepatic production of the egg yolk protein, vitellogenin, normally induced by endogenous estrogen. Channel catfish were fed a specialized diet containing can- sein as the protein source, and not containing soy, for 18 days to allow dissipation of genisteen from the tissues. Channel catfish were administered 0.2mg/g 17 beta-estradiol, as a positive control, and serum was collected. Vitellogenin was precipitated using N, N-dimethylformamide from serum of catfish treated with 0.2mg/g 17 beta-estradiol. Total protein in the precipitate was determined using the BCA protein assay. Vitellogenin was identified using Western blot and quantified using ELISA with a vitellogenin specific monoclonal antibody. Carcass were gavaged once with 0.2mg/g, 1.0mg/g, or 2.0mg/g body wt genisteen. Serum and liver samples were taken for analysis. A dose-response curve for vitellogenin synthesis was generated from catfish gavaged with genisteen. A second group was gavaged with 0.2mg/g 17 beta-estradiol. A third group was gavaged with 0.2mg/g 17 beta-estradiol to determine the dissipation of genisteen in the liver and the effect of genisteen on vitellogenin synthesis over time. A third group was placed on a soy-based diet. Levels of vitellogenin in the liver were determined by HPLC. The fish that were gavaged with genisteen or placed back on a soy diet showed an increase in vitellogenin in the liver. Soy fed catfish had vitellogenin levels in their serum greater than casein fed catfish. The results suggest that the phytoestrogens present in commercial soy-based catfish feed can bind the estrogen receptor and induce the synthesis of vitellogenin. (Supported by USDA/ARS 58-0790-6-120).
1688  THE VALUE OF LACTIC ACIDOSIS IN THE ASSESSMENT OF THE SEVERITY OF ACUTE CYANIDE POISONING.

P.J. Baudu, B. Mégarbane, S. W. Boron and C. Bismuth. Intensive Care Unit, Lariboisière Hospital, Paris, France.

Introduction: In pure cyanide poisonings, the correlation between plasma lactate concentrations and the corresponding blood cyanide concentrations has not been addressed. Thus, we tested the hypothesis that the repeated measurement of plasma lactate concentrations could have a confirmatory value in acute pure cyanide poisonings. Materials and Methods: We reviewed the charts of the 11 patients admitted to our toxicological intensive care unit between 1988 and 2000 who had exposures to cyanide. Victims of smoke inhalation were excluded from this study. Ten patients had pre-existing blood samples. Results: Blood lactate and plasma lactate concentrations were measured by a colorimetric assay using microdilution and an enzymatic method (Nak 9 to 18 mg/dl, respectively). Results: Before antitoxaphen treatment, the median plasma lactate concentration was 168 mg/dl, the median blood lactate concentration was 4.2 mg/dl. Using Spearman test, there was a significant correlation between plasma lactate and blood lactate concentrations (r = -0.74, p = 0.017). Before antitoxaphen treatment, plasma lactate concentration correlated positively with anion gap and inversely with systolic blood pressure, systolic blood pressure, systolic arterial pH, and cyanide blood concentrations correlated inversely with anion gap and blood concentration. During the course of cyanide poisonings, a plasma lactate concentration > 72 mg/dl (8 mmol/l) was sensitive (94%) and moderately specific (70%) for a toxic blood cyanide concentration (>1.0 mg/l). The specificity was substantially improved in patients not receiving catecholamines (85%). Conclusions: The immediate and serial measurement of plasma lactate concentration is useful in assessing the severity of cyanide poisoning.

1689  CONSEQUENCES OF BRIEF EXPOSURE TO HIGH-CONCENTRATION CARBON MONOXIDE IN AWAKE RATS.


Exposure to high concentrations of carbon monoxide (CO) is of concern in military operations. Experimentally, the harmful consequences of a brief exposure to high concentration CO have been poorly described. This study investigated the development of acute CO poisoning in awake male Sprague-Dawley rats (220-300g). Animals were randomly grouped and exposed to either 1000, 3000, 6000, 10000, 12000 or 24000 ppm CO in a continuous air/gas dynamic exposure chamber for five minutes. Respiration was recorded prior to and during exposures. Mixed blood carboxyhemoglobin (COHb) and pH were measured before and after exposure. Compared to the pre-exposure control, at the end of the exposure to 1000, 12000, and 24000 ppm groups, breath rate was significantly decreased by 64%, 47%, and 18%, respectively. Tidal volume was significantly increased by 121, 137, and 142%, and minute ventilation volume was significantly decreased by 77, 64, and 26%, respectively. Blood COHb was elevated to 60.1, 63.4, and 69.3%, and blood pH was reduced to 7.43a 0.09, 7.25a 0.05, and 7.13a 0.04, respectively. The mortality was 1/12 in the 24000 ppm group and 4/12 in the 24000 ppm group. Deaths occurred in the fifth minute of exposure. Breath rate was < 27% of the control, pH < 6.87, and COHb > 80% in animals that died during exposure. There were no significant changes in respiratory parameters and pH, and no deaths, in rats exposed to CO concentrations at 6000 ppm, although COHb was elevated to 14.5, 29.7, and 54.3% in the 1000, 3000, 6000 ppm groups, respectively. These results suggest that brief exposure to CO at concentrations under 10000 ppm may cause minimal to moderate health risk. However, exposure to CO at concentrations greater than 10000 ppm for a period of time as short as five minutes will change breathing patterns which may result in acute respiratory failure, acidemia, and even death.

1690  AN OUTBREAK OF HEPATITIS AS A RESULT OF OCCUPATIONAL EXPOSURE TO SOLVENTS IN A HAZARDOUS WASTE FUEL BLENDING FACILITY.


This is a multi-disciplinary investigation of an outbreak of hepatitis. The methods included environmental and occupational health investigations, epidemiological survey, clinical examination, liver function tests, liver biopsy, serological tests for hepatitis A, B, and C, and serum and/or liver biopsy. The study included 91 workers tested, 44% exhibited symptoms of hepatitis consistent with exposure to chlorinated solvents. These symptoms included nausea, vomiting, abdominal pain, diarrhea, anorexia, weight loss, and hepatitis A levels higher than 1.5 times normal were recorded as case. Among the cases 13% tested positive for hepatitis B or C. A total of 92% of these cases worked in the tower where the fire and clean up occurred. All maintenance workers participating in repair and clean-up had elevated ALT. The liver biopsy of the worker with the highest ALT (8,000 IU) indicated diffuse hepatitis with acidophilic bodies and microvesicular fat on oil red O stain. Among the workers not normally working in the tower, only 10% had cases. After comparing the medical history, test results, primary work locations, job descriptions, onset of symptoms, usage of PPE among the workers, and other health and safety measures, it is concluded that the outbreak of hepatitis probably resulted from exposure to solvents during the post-fire cleanup. The identification of chloroform and coluranics by serum infrared spectroscopy in one case, and the presence of chlorinated compounds in the fire retardant surfactant suggest that chloroform/carbonates may have been liberated leading to inhalation exposure as a result of heat from the fire.

1691  COMPARATIVE OCCUPATIONAL EXPOSURES TO FORMALDEHYDE RELEASED FROM INHALED WOOD DUSTS VERSUS THAT IN GASEOUS FORM.

N. H. Gosselin, R. C. Brunet and C. Carrier. *Santé environnementale et santé au travail, Université de Montréal, Montréal, PQ, Canada; and **Mathématiques et Sciences de l'Ingénierie, Université de Montréal, Montréal, PQ, Canada.

Particle boards and other wood boards are usually made with formaldehyde-based resins. Wood workers are thus exposed to formaldehyde in gaseous form as well as from airborne dust once it enters their respiratory tract. These workers remain exposed to formaldehyde released from the dust still present in their upper respiratory tract, even after their work shift. In assessing the risk associated with formaldehyde exposure, one needs to consider the relative importance of these two sources of exposure. This study proposes a kinetic model to estimate the exposure to formaldehyde released from dust and compares it to exposure from the gaseous form. Model parameters are determined using data from published studies which estimate amounts of dust deposited in different regions of the respiratory tract. Our results indicate that most of the formaldehyde released from wood products is dust once in the trach. The model predicts the amount of formaldehyde absorbed by the respiratory tract upon its release from the wood product dust for various exposure scenarios. Based on a daily work shift of 8 hr, it is found that, for a dust concentration in air of 5 mg/m3 and a formaldehyde concentration of 9 mg/m3 of dust, the amount of absorbed formaldehyde released from wood dust is approximately 1/100 of the amount absorbed from the gaseous form at a concentration level of 0.38 mg/m3 (0.3 ppm). Considering that the formaldehyde concentration in wood dust used above is much higher than usually observed and that dust and gaseous form formaldehyde concentrations used are of the order of the recommended threshold limit values, the simulations show that the formaldehyde exposure from wood dust is comparatively negligible.

1692  ASSESSMENT OF TOluene AND ETHYL ACETate EXPOSURE FROM WEARING A COSTUME MASK BONDED WITH CONTACT CEMENT.

M. J. Fedorka and B. D. Keager. *Center for Occupational & Environmental Health, University of California, Irvine, Irvine, CA and **Health Science Resource Integration, Inc., Tallahassee, FL.

A series of experiments was performed to assess the solvent evaporation rates and relevant exposure concentrations for primary solvents (toluene and ethyl acetate) emitted from a contact cement used to bond foam surfaces within a costume mask (a furry bear head) worn by theatrical dancers. Four areas of the contact cement were applied to each of four 3 by 3 inch areas on both foam components of
the mask (the fitted helmet and the mask structural foam); each coat was allowed to dry for 10 minutes before bonding (joining the cement-coated foam surfaces). Solvent concentrations were measured within the mask under static conditions at 2, 4, 8, 16, and 20 hours after bonding. Each sample was collected as a one-minute grab sample using 1 liter Summa canisters, and analyzed by EPA Method TO-14. An exponential loss pattern was observed for both toluene and ethyl acetate concentrations inside the mask. Toluene acetate levels diminished quickly and were nondetectable (1.2 ppm) at 8 hours, while toluene levels diminished quickly but were stable at 11 to 14 ppm when measured at 8, 16, and 20 hours after bonding. Personal toluene exposure was simulated at 21 hours after bonding by fitting the mask on an adult-size mannequin equipped with a breathing zone sampling port in a convection chamber at 37°C. Toluene exposure concentrations in the breathing zone were 1.3-2.9 ppm when measured at 5, 10, 15 and 20 minutes after donning the mask, and a level of 7 ppm was observed at 30 minutes. These studies illustrate that the majority of solvents in contact cement were evaporated from the costume mask under static conditions within 4 to 8 hours after bonding, that residual toluene levels after that time are relatively low, and that donning the mask displaces most of the residual toluene. Toluene exposures at 1 to 7 ppm are well below occupational exposure limits and adverse effect levels in humans.

1693 AMMONIA EXPOSURE AND DOSE-RESPONSE ASSESSMENT FOR HOUSEHOLD CLEANING USES.

B. D. Kerger and M. J. Federok.

There is scant information pertaining to airborne ammonia exposures from common household use of ammonia-containing floor/tiler cleaners and spray-on glass cleaners. We assessed instantaneous and event-specific time-weighted average (TWA) exposures to airborne ammonia during mixing and use (cleaning with a soaped sponge) of a typical household floor/tile cleaner containing 5% ammonium hydroxide. One cup of the ammonia cleaner was mixed with two gallons of hot water to make the washing solution, as per the label directions. Airborne ammonia sampling was conducted while the tile walls and floors within three different size bathrooms in a residence were washed. We also assessed exposures during use of a spray-on glass cleaner containing 1 to 3% ammonium hydroxide, while washing several large windows in a small office setting. In each case, TWA ammonia was assessed using a breathing zone personal sampling pump equipped with a sili gel tube and analyzed by NIOSH Method 6015. Simultaneous measurements were recorded each minute with a breathing zone probe from a Drager PAC III monitor with electrochemical cell detector. Peak ammonia levels ranged from 16 to 28 ppm and short-term TWA concentrations ranged from 3.4 to 13 ppm during mixing and cleaning in each of the three bathrooms. Ammonia exposures while using spray-on window cleaners were below reliable detection limits (monitor <3 ppm; TWA <1.3 ppm). Brief, moderate exposure levels for airborne ammonia can occur during standard bathroom cleaning uses, i.e., averaging 11 ppm. Peak ammonia concentrations were the highest during the TWA occupational exposure limit (OEI) of 25 ppm. Use of the concentrate or less dilute cleaning solutions could result in ammonia exposures exceeding the OEL ceiling of 50 ppm, and could cause moderate eye, nose and throat irritation and respiratory symptoms in sensitive individuals. Thus, ammonia-containing cleaner products should contain clear warnings to avoid use of undiluted concentrates.

1694 EXPOSURE OF CHILDREN AND ADULTS TO TRANSFERABLE RESIDUES OF CHLORPYRIFOS FROM DOGS TREATED WITH FLEA CONTROL COLLARS.

J. S. Booze, J. Tyler and J. E. Chambers.

Twenty-four households were chosen that included a pet dog and a child between the ages of 3 and 12 years. These family units were used to determine the levels of insecticide that could be transferred by a flea control collar from a pet dog and any increased amounts of chlorpyrifos metabolites in the urine of one child and one adult in the household. Commercially available flea control collars containing the organophosphate insecticide chlorpyrifos (8%), 2.54g were applied to dogs as per package directions. Transferable residues were quantified by peting the dog in 3 areas (back, neck, neck with collar) with cotton gloves for 5 min and analyzed by GC/ECD. The amount of chlorpyrifos residues transferred were highest from the thighs contacting the flea collar (168-454) mg, lower from the collar (49-252) mg, and lowest from the fur of the back distant from the collar (3-14) mg. The urinary metabolite 3, 5, 6-trichloro-2-pyridinol (TCP) was quantified in first morning void samples using a magnetic particle/antibody assay. The urine of both adult and children obtained before the collar was placed on the dog showed measurable levels of TCP, presumably because of the numerous approved agricultural and residential uses of chlorpyrifos. Ranges of urinary TCP levels in the adults and children prior to placement of the flea collar on the pet dog were 1.3-35.0 and 1.9-22.4 ng/ml, respectively. There were no statistically significant increases in urinary TCP placebo adults or children for 6 to 8 weeks after application of the flea collar. The range of levels in the adults and children after application of the flea collar to the dogs were 1.4-52.0 ng/ml and 1.2-52.4 ng/ml, respectively. These results suggest that the insecticide residues obtained from the routine use of a flea collar did not contribute significantly higher levels of exposure to the people in contact with pet dogs. (Supported by EPA R 825170.)

1695 MEASURING AND ASSESSING THE RISK OF CHEMICAL EMISSIONS FROM CONSUMER PRODUCTS: SUMMARY STUDY OF OFFICE PRINTERS.

D. R. Cortes, M. S. Black and A. G. Wortham.

Air Quality Sciences, Marietta, GA. Sponsor: K. Willett.

Good product stewardship as well as regulatory and non-regulatory programs necessitates knowledge of high and low-level chemical emissions from consumer products, and understanding their risks to users. This requires a complete IAQ exposure assessment that includes multiple components. This study summarizes the results of the assessment of over 100 office printers. Testing was conducted under environmentally controlled chamber conditions with parameters appropriate for the intended use of the product. A sensitive sampling and analysis method with a specialized chromatographic and mass spectral indoor air library was used to properly identify all emissions. Because accurate emission rates are needed to predict consumer exposures for comparison to specifications set by government and private organizations, product-specific emission profiles were fit to appropriate mathematical models. The predicted user exposure concentrations were then compared to multiple databases containing information on inhalation health risks and irritation/odor potentials of all of the chemicals identified in the product emissions. The most common emissions from laser printers were styrene, xylene, and ethylbenzene, while the most common emissions from plotters were acetic acid, benzyl alcohol, and nonanal. These types of compounds are fairly typical in the indoor environment, and the exposure levels contributed by these emissions are low relative to normal indoor levels. However, the compound list is different when examined in terms of highest average emissions. For laser printers they are butyroligol (emission rate - 11, 305 µg/hr), cyclohexane (6, 055 µg/hr), and 2, 2, 4, 4-tetramethylpentane (3, 500 µg/hr), while for plotters they are 2-propanol (19, 500 µg/hr), ethanol (8, 000 µg/hr), and 1, 3-dichloro-2-propanol (3, 800 µg/hr). Exposure concentrations of these and hundreds other chemical emissions were calculated based on a typical use scenario, and then compared to multiple databases to determine health risk, discomfort levels, sensory irritation, and odor perception.

1696 ESTIMATION OF DIETARY INTAKE OF INORGANIC ARSENIC IN CHILDREN.

L. J. Yost, R. S. Schoof, and M. R. Garr.

Exponent, Bellevue, WA. "Gradient Corporation, Bellevue, WA and Exponent, Bellevue, WA.

Arsenic is a natural component of our environment, and is ubiquitous in soils and in the diet. Accurate dietary intake estimates for inorganic arsenic are needed to establish dietary intake of inorganic arsenic from exposure of children. Previous investigations have estimated dietary intake of inorganic arsenic from exposure in adults ranging from 1 to 20 µg/day with an average of 3.2 µg/day, based on a comprehensive market basket survey in which 40 commodities anticipated to provide at least 50 percent of dietary inorganic arsenic intake were analyzed for inorganic arsenic content (Schoof et al. 1999a, b). Four samples of each commodity were collected. Total arsenic was analyzed using an atomic absorption spectrometry/field. Selected aliquots were analyzed for arsenic species using HCl digestion and ICP atomic absorption spectrometry (Schoof et al. 1999a). Application of total arsenic intake data from FDA for the years 1982 to 1991, with recent inorganic arsenic percent resulted in an estimate of average dietary inorganic arsenic intake for children (ages six months to two years) 6.7 µg/day. Because inorganic arsenic in the diet is naturally occurring and exposure unavoidable, this intake estimate provides a useful context in risk assessment of arsenic exposure.
RISK CHARACTERIZATION USING DIFFERENT ASSUMPTIONS REGARDING SPECIATED ARSENIC IN FISH AND SHELLFISH.

R. Lorenzo\(1\), D. Davoli\(2\), A. Johnson\(3\) and H. Cloudshar\(3\)
\(1\)Region 10, USEPA, Seattle, WA. \(2\)Environmental Assessment, Washington Department of Ecology, Olympia, WA. \(3\)ORD-NCEA, USEPA, Cincinnati, WA.

When total arsenic tissue concentrations are available, assumptions are required regarding the concentrations of speciated arsenic in fish and shellfish. Fish and shellfish data for the Pacific Northwest are included. The concentrations of different species in the fish and shellfish are determined. This work demonstrates the effect of different assumptions on the total arsenic in fish and shellfish recently collected for freshwater and saltwater species in the same area. The results of a worldwide literature search provide the basis for initial assumptions. Additionally, assumptions based on a small amount of speciated arsenic data from the Pacific Northwest are included. Total arsenic concentrations are higher in freshwater fish than in freshwater fish. Based on sparse information, it appears that the trophic level fish tend to have higher concentration of arsenic. Total arsenic concentrations tend to be higher in shellfish than in fish, with a great deal of species variability. The percentage of arsenic in shellfish is higher than in fish, as is the percentage of another toxic arsenical species, dimethylarsinic acid. Uptake of arsenic in an arsenic-contaminated system appears to be a complex process, and no generalizable trends were observed in the data. In an evaluation that assesses risks from multiple contaminants in the same animal species, the impact of the percent arsenic on the overall contaminant concentration present in the species. The importance of the amount of arsenic in animals increases as all contaminant concentrations decrease. While having specific arsenic data is preferable in all situations, it is most critical when total contaminant concentrations are suspected to be low. Use of speciated arsenic data facilitates risk management decisions by avoiding risk characterizations in which arsenic is based on assumptions rather than measurements, predetermines the risk.

ESTIMATION OF INORGANIC ARSENIC INTAKE FROM FISH-MARKET BASKET VS RECREATIONAL CATCHES.

R. A. Schoof\(1\) and L. J. Yost\(1\)
\(1\)Closed Corporation, Mercer Island, WA and \(2\)Exponent, Bellevue, WA.

Seafood contributes the greatest fraction of total As to the diet; however, the major fraction of As in fish is composed of complex organic arsenicals. The contribution of fish to dietary intake of the more toxic inorganic As(III) is pertinent both for assessing background As exposures and for assessing the contribution of As in recreationally caught fish to As exposures from surface water. Due to the expense and difficulty of analyzing As concentrations in fish, most available fish data are for total As only. Dietary intake estimates for fish are frequently made by assuming that a certain fraction of the As reported in the fish is As(III). For example, an assumed 10% of seafood As is inorganic. Limited data has been available to support such assumptions, but several recent studies suggest that the NRC (1999) assumption may be too high. Schoof et al. (1998) reported data for saltwater fish, As(III) in, 30% of the total As in marine fish is inorganic. The As(III) in all these samples was very low, so the variation in the As(III) inorganic content is almost solely due to variations in the amount of As(III) in. Bercht et al. (1994) reported a somewhat higher As(III) content in marine fish, ranging from 0.4 to 3%. Since most seafood intake in typical diets is from marine sources, these other recent studies suggest that the NRC assumption is an overestimate, and that As(III) is closer to 1% of As in seafood. New data also allow estimates of fraction of As in wild freshwater fish. EVS (2000) report As(III) and As for fish caught in the Willamette River in Oregon. No As was detected in pike minnow, while the fraction of As(III), which was As(III) averaged 5%, 4%, and 15% in bass, carp, and suckers, respectively. The implications of these findings for risk assessment of As in fish in surface water and the derivation of ambient water quality criteria for As will be explored.

DOSE RECONSTRUCTION OF BENZENE EXPOSURE FOR PLOIFILM COHORT (1936-1976) USING MONTE CARLO TECHNIQUES.

P. Williams and D. Paustenbach
Exponent, Mendo Park, CA.

Regulatory standards or guidelines for occupational exposure to benzene are based predominantly on studies of workers. The cohort of rubber hydrochloride (Pliofilm) workers, in particular, serve as the primary basis for the NIOSH Permissible Exposure Limit (PEL) and ACGIH Threshold Limit Value (TLV). Previous assessments of this cohort by Rinsky (1981), Crump and Allen (1984), and Paustenbach et al. (1992) were based on varying assumptions about workplace practices and processes over time, as well as different exposure data and assumptions, thereby yielding significantly different estimates of benzene dose for certain job categories. Given the inherent uncertainties and uncertainties involved in estimating historic exposures for this cohort, a "distributitional" rather than a "point estimate" approach should be more informative for this cohort. In this paper, we reevaluate the Pliofilm data using Monte Carlo techniques and discuss the impacts of various exposure parameters on estimated doses. A key objective of this work is to address criticisms that previous exposure-ratio parameter values were either too high or too low. Estimated benzene doses are reported as equivalent 8-hour time-weighted averages for more than ten job categories from 1936 to 1965 (Akon and Ilaa facilities) and 1940 to 1976 (St. Marys facility). The key sources of uncertainty addressed in the analysis include characterization of work area configuration (backyard and peak), uptake of benzene from dermal exposures, engineering controls over time, extended work hours and plant shut down in early years, accuracy of monitoring devices, and effectiveness of personal protective equipment. Sensitivity analyses are used to identify areas of uncertainty that have the greatest impact on estimated benzene dose. The results of this analysis provide more thorough and defendable characterization of benzene exposures among the Pliofilm cohort than those previously reported. These results may also be used, in conjunction with the available mortality data, to provide a more refined assessment of benzene's chronic toxicity in humans.

RATE OF HEXAVALENT CHROMIUM REDUCTION BY HUMAN GASTRIC FLUID.

D. M. Posner, P. M. Harv, V. V. Ruby, S. Lin, A. Sjog, M. Goodman and D. J. Paustenbach
Exponent, Irvine, CA. Exponent, Boulder, CO and Exponent, Mendo Park, CA.

Hexavalent chromium [Cr(VI)] is reduced to the essentially non-toxic trivalent state [Cr(III)] in the human stomach. This study investigates the rate of Cr(VI) reduction under a variety of simulated gastric conditions, and in real human gastric juice collected from fasting volunteers. The rate of Cr(VI) reduction in simulated gastric fluid was measured at pH values from 1.5 to 4.5, and in Cr(VI) concentrations ranging from 0.1 to 4.5 ppb, in diluted stomach fluid (10-fold, following ingestion of Rotella, and in the presence of food. Stomach conditions were simulated by preparing an aqueous mixture of the primary components of gastric fluid (pepsin, mucin, gastric lipase, gastric amylase, etc.), acidifying with HCl, and providing continuous stirring and heating to approximately 37°C. All experiments were conducted for 60 minutes, with Cr(VI) measured using a Hach AccuV. Gastric ChromaVer instantaneous test kit. The findings of these studies are: 1) real human stomach fluid has a 10-fold greater capacity to reduce Cr(VI) than simulated stomach fluid; 2) within the first 2 to 5 minutes, 0.3 to 1 mg Cr(VI)/L is reduced in real stomach fluid collected under fasting conditions; 3) increasing gastric pH from 1.5 to 4.5 reduces the rate and extent of Cr(VI) reduction by at least one-third; 4) the amount and rate of Cr(VI) reduction is constant regardless of the starting Cr(VI) concentration, which suggests that these results may be extrapolated to lower concentrations of Cr(VI) in drinking water; 5) antacids dramatically increase the pH of the stomach (from pH 1.5 to 8.2) but appear to have little effect on the Cr(VI) reduction rate or capacity for reduction at pH 4.5; and 6) the presence of food substantially increases Cr(VI) reduction in simulated stomach fluid, with 10 mg/L reduced within the first 4 minutes. These results suggest that Cr(VI) ingested in the field MCL of 100 ppb, would exist in the stomach, even under fasting conditions, for less than 1 minute before being reduced entirely to Cr(III).

EXACERBATION OF UROPHYLLA DEVELOPMENT IN MICE HOMOZYGOUS FOR GENETIC DISRUPTION AT THE HEMOCROMATOSIS (HFE) GENE.

M. R. Franklin\(1\), J. D. Phillips\(1\) and J. P. Kuthner\(1\)
\(1\)Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT and \(2\)Division of Hematology, Department of Medicine, University of Utah, Salt Lake City, UT.

Two recently developed strains of genetically modified mice have made it possible to study the contribution of mutations at the hemochromatosis (Hfe) and uroporphyrinogen decarboxylase (URO-D) genes to porphyria susceptibility. Homozygosity for the null mutation of the Hfe gene (URO-D-J) was lethal. Homozygosity for the null mutation of the Hfe gene caused high levels of iron to accumulate in the liver; homozygosity was without effect. Neither URO-D nor Hfe gene disruption alone was sufficient to cause the development of uroporphyrin. We have investigated polyallogenic with the transgenic mouse model, as each of these two genes are necessary for the development of uroporphyrin. In mice injected with a transgenic uroporphyrin gene, a both these genes are necessary for the development of uroporphyrin. In mice injected with a transgenic uroporphyrin gene, the presence of high hepatic iron load from a disruption of the hemochromatosis gene in addition exacerbates the porphyric response. The hepatic porphyrin
levels were higher in female mice. Mice genetically normal at the urorophyrinogen deacetylase gene locus do not develop urorophyrinogen deacetylase activity determined in subsequently isolated cytosol, and upon heat denaturation of that cytosol, the presence of a factor that inhibits recombinant human urorophyrinogen deacetylase enzyme. Overall, hepatic siderosis from Fe gene disruption in mouse models of porphyria cutanea tarda was insufficient to cause urorophyrin, but exacerbated its development in mice made susceptible through genetic depression of constitutive URO-D activity.

1704 ENZYMATIC OXIDATION OF PCB METABOLITES INCREASES INTRACELLULAR OXIDATIVE STRESS.
A. Shinuyan, P. Espandari, L. W. Robertson and G. Ludewig, Graduate Center for Toxicology, Univ. of Kentucky, Lexington, KY.
Polychlorinated biphenyls (PCBs) are highly persistent environmental contaminants. Questions remain concerning the mechanism(s) of PCB carcinogenicity. PCBs are metabolized to mono- and dihydroxylated compounds. In vitro the dihydroxylated compounds are further metabolized either slowly by autooxidation or rapidly in the presence of enzymes like peroxidases or prostaglandin synthase to quinones, accompanied by the generation of reactive oxygen species (ROS). In this study, we wanted to evaluate the effect of enzymatic oxidation of PCB metabolites in cells in culture in terms of ROS production. Three human cell lines with different metabolic activities were chosen for this analysis: the leukemia cell line HL-60 (high in myeloperoxidase), the breast cancer cell line MCF-7 (high in prostaglandin synthase) and the lymphoma cell line Jurkat (low levels of either enzyme). We used the hydroquinone and para-quinone of 4-chlorobiphenyl (4CPH-HQ and 4CPH-pQ), respectively, as test compounds. Intracellular ROS production was determined using dichlorofluorescin-diacetate (DCFH-DA) that is converted by cellular esterases to DCFH and further in the presence of ROS to the fluorescent DCF, which can be detected by fluorometry, flow cytometry or confocal microscopy. Propidium iodide was used to measure viability. 4CPH-pQ caused a dose-dependent increase in fluorescence in all three cell lines at sub-lethal concentrations. With 4CPH-HQ, however, treatment of MCF-7 cells produced an increase in intracellular fluorescence while it had no effect on Jurkat cells. Cells were mostly viable in all treatments with the exception of HL-60 cells in the presence of ≥10 μM 4CPH-pQ. These results show that PCB quinones can produce intracellular oxidative stress in any cell type. On the other hand, the conversion of quinone to quinone and hence ROS production occurs at a higher rate only in cell types with high amounts of peroxidases or prostaglandin synthase (Supported by ES 07380).

1705 EFFECT OF PCB 77 ON TOTAL SELENIUM AND SELENIUM-DEPENDENT GLUTATHIONE PEROXIDASE IN RAT LIVER TISSUES.
D. N. Stemn, T. P. Twigg, P. Espandari and L. W. Robertson, Graduate Center for Toxicology, University of Kentucky, Lexington, KY.
Selenium (Se) provides defense against oxidative damage and is a critical component of several proteins including glutathione peroxidase (SeGPx), a cellular antioxidant enzyme. Persistent inducers of cytochrome P450, such as the polychlorinated biphenyls (PCBs), may generate reactive oxygen species and are associated with oxidative stress. Among the PCB congeners, PCB 77 (3, 3', 4, 4', tetrachlorobiphenyl) is an aryl hydrocarbon receptor (AhR) agonist, and an efficacious inducer of cytochrome P450. The effect of PCB 77 on hepatic total Se and SePpx activity in male and female rats was investigated. We administered PCB 77 dissolved in corn oil (300μg/kg i.p., bolus dose) to male and female Sprague-Dawley rats fed with refined rat chow. The animals were sacrificed at different time points after dosing: 6 hours, 12 hours and 1, 4, 7, 14 and 21 days. Cytochrome P450 1A1 was induced in all treated animals. Hepatic total Se levels was decreased by 40% to 21 days treatment groups of female rats. In contrast, there was no marked reduction in the treated male rats. Hepatic, cytosolic SeGPx activity was also diminished by 40% to 21 days time points for both male and female with the 3 week time point reduced by 6 times. However, the female SeGPx activity was markedly higher than the male in all time points except for the 12 hour time point. A study of GPx activity in vehicle (corn oil) treated rats found that GPx activity in the liver were higher in female than male rats. This indicates that female rats may be more sensitive to PCB 77 effects on Se. Gender also appears to influence PCB 77 associated effects on the SeGPx enzymatic activity. (Supported by ES 07380).

1706 DNA ADDUCT FORMATION BY POLYCHLORINATED BIPHENYLS: CROSS-SPECIES COMPARISON OF MICROSOMAL BIOACTIVATION SYSTEMS AND ADDUCTION BY SYNTHETIC QUINONE METABOLITES.
D. Perez-Parce, L. W. Robertson1 and R. C. Gupta1, Graduate Center for Toxicology, University of Kentucky, Lexington, KY. Preventive Medicine and Environmental Health. University of Kentucky, Lexington, KY and Health and Environment Unit, Naval University Medical Research Center, Ste. Foy, PQ, Canada.
PCBs are ubiquitous environmental contaminants. They are complete carcinogens in rodents and DNA adduction by PCBs was reported. The current study was designed to compare DNA adducts formed after bioactivation of PCBs with rat, mouse and human hepatic microsomes, and to investigate the role of quinoid PCB metabolites in DNA adduction. Eight congeners ranging from mono- to hexachlo-

1703 GLUCURONIDATION OF HYDOXYLATED POLYCHLORINATED BIPHENYLS.
N. M. Tampal, H. J. Lehmler, P. E. Espandari and L. W. Robertson, Toxicology, University of Kentucky, Lexington, KY.
Polychlorinated biphenyls (PCBs) are metabolized to hydroxylated compounds. While many of these metabolites are further converted to either the glucuronic acid or sulfate conjugates by phase II enzymes, which facilitates their excretion, some hydroxylated PCBs persist in the body, reflecting their inability to be conjugated. A possible role of uridine diphosphate glucuronyl transferase (UDPGT) in the elimination of hydroxylated metabolites of PCBs was investigated. Glucuronidation studies of some PCB metabolites, including ones which are eliminated with relative ease and also ones which are reported to be retained in blood, were carried out. Liver microsomes, prepared from male Wistar rats treated by intraperitoneal injections of phenobarbital for 3 days (400 μmol/kg/day), were used as the source of UDPGT. Enzyme kinetic (Vmax/Km) were determined for each of the metabolites. The enzyme efficiency towards glucuronidation (Km/Kmax) was found to be dependent on the structure of the substrates. Substitution of chlorine atoms on the non-hydroxylated ring greatly lowered the Vmax of the enzyme, with substitution in the meta- and para-positions being least favorable for enzyme activity. Steric hindrance around the hydroxyl group by chlorines on adjacent carbon atoms did not play a major role. Also, no apparent correlation between the calculated dihedral angle (planarly) and enzyme activity was determined (r2 < 0.5) however, a weak correlation for the shape area of the molecule and partition coefficient was observed (r2 > 0.5). (Supported by P42 ES 07380).
1707

THE EFFECTS OF PURIFIED VS. UNREFINED DIETS ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN TESTES OF PCB-153-TREATED RATS.

Z. A. Feidhel1, N. Stafins1, H. P. Glaeser1, L. W. Robertson1 and G. Ludewig2

1University of Petra, Amman, Jordan and 2University of Kentucky, Lexington, KY.

The tests are known to be highly sensitive to a number of physical stresses. Among others, reactive oxygen species (ROS) have been shown to impair testicular function. Previous observations led us to postulate that different diets may influence oxidative stress levels in the testes. In this study we compared the effects of purified vs. unrefined diets on 1) the production of thiobarbituric acid-reactive substances (TBARS) in the testes as an indicator of oxidative stress, and 2) the activities of antioxidant enzymes in the testes. Thirty-six male Sprague Dawley rats were fed a purified diet (similar to the AIN-93M diet) or an unrefined diet (Teklad Global 19% Protein Rodent Diet) for 3 weeks. Six animals per diet received a single IP injection of 2, 4, 4', 5', 5'-hexachlorobiphenyl (PCB-153, 300 µmol/kg), while six other animals per diet received no injection (control) or the vehicle only (corn oil). All animals were euthanized 2 days after treatment, the testes were excised, and subcellular fractions were prepared by differential centrifugation. The results indicate that feeding a purified diet to rats produced a significant decline in TBARS in testes cytosol in control and vehicle groups, and in animals treated with PCB-153 compared to animals on unrefined diets. No reduction in TBARS was seen in testes homogenates. Catalase activity in testes was increased in control groups receiving purified diet compared to unrefined diet, but not in PCB-treated groups. Glutathione transferase activity gave mixed results and total glutathione peroxidase activity was unchanged. This study shows that purified diets may lower oxidative stress in rat testes and that catalase activity may be partially responsible for this decrease. Further studies are needed to identify those dietary components responsible for these phenomena. (Supported by NIH ES07380 and EPA COR20395).

1708

DOES AN ENVIRONMENTAL PCB/PCDF MIXTURE ENHANCE ULTRAVIOLET RADIATION-INDUCED SKIN CANCER?


The incidence of non-melanoma skin cancer (NMSC) has been increasing significantly. Chemical carcinogens synergistically interact with solar ultraviolet radiation (UVR) in non-melanoma skin cancer development. PCBs/PCDFs are ubiquitous environmental contaminants and potential epidermal carcinogens that bioaccumulate in skin. Our objective was to examine the interaction between environmental and genotoxic chemicals and UVR on skin carcinogenesis. Our aim was to test the hypothesis that living in a PCB/PCDF contaminated area would enhance UVR-induced skin carcinogenesis in the C3H/SH-Het mouse model of human NMSC. Four groups of mice were placed on either PCB/PCDF contaminated soil collected from an old electric landfill in Illinois or control soil for weeks 1-11. Mice were weighed weekly. Two weeks after the beginning of the experiment, 2/4 groups of mice, 1 on treated and 1 on control soil bedding, were exposed to UVR 5 days/week. Mice exposed to the chlorinated aromatic contaminated soil were significantly (p<0.001) heavier than those exposed to control soil. At week 18, papillomas were only present in the chemical mixture treated groups. Regardless of UV exposure, the tumor incidence and multiplicity were significantly greater in the UVR/contaminated soil treated group compared with the no UVR/contaminated soil treated group. Based on the preliminary results, a synergistic interaction between sun and UVR on skin carcinogenesis is likely. Therefore, environmental exposure to chemical carcinogens could possibly be an important factor that enhances the incidence of NMSC.

1709

EFFECTS OF POLYCHLORINATED BIPHENYLS AND 2, 3, 7, 8-TETRACHLOROBENZO-α-DI-OXON ON PROLIFERATION OF "CELL CYCLE PROLIFERATION REPRESSION IN HUMAN PLACENTAL TROPHOBLASTIC JEG-3 CELLS.


The environmental contaminants polychlorinated biphenyls (PCBs), dioxin and polynonyromic hydrocarbons have been linked to infertility and low birth weight in animals and humans. This study used the JEG-3 human placental trophoblast cell line to investigate potential growth inhibitory effects of prototype organochlorines in relation to aryl hydrocarbon receptor (AhR) mediated induction of cytochrome PaS 1A1. Cells were treated respectively with the AhR ligands 2,3,7,8-tetrachlorobiphenyl (Aroclor 1254) (TCDD), BENZO(A)PYRENE (BaP) and 3,3', 4', 4'-tetra-CB (TCB) in comparison with 2,2', 4, 4', 5, 5-hexachlorobenzene (HCB), which is not an AhR ligand. Five-day treatment in the presence of 10% serum, mitomycin C and 10 M BaP were used to reverse the treatment. Following five-day treatment in the presence of 10% serum, mitomycin C and 10 M BaP were used to reverse the treatment.

1711

CAUSATION ANALYSIS EXPOSES CRITICAL WEAKNESSES IN PURPORTED LINK BETWEEN PCBs AND NEURODEVELOPMENTAL DEFICITS.

J. B. Silwok,1 J. E. Bernier,1 J. Borak1 and D. Palumbo.1 GE Corp. Res. & Dev., Schenectady, NY; State University of New York, Albany, NY; Yale School of Medicine, New Haven, CT and UMass of Boston, Boston, MA.

Causation analysis was applied to 24 epidemiological studies that sought an association between maternal PCB exposure and neurodevelopmental effects in 2,573 children in 6 international cohorts. Our analysis judged: strength of association; consistency; evidence of a dose-response relationship; specificity of effect; biological plausibility; and temporality. Across the epidemiology studies, 5 analytical approaches were used to measure PCBs in biologic media. Results involved more than 60 PCB metrics (including statistically generated composites) to assess exposure. Neurodevelopmental status was evaluated from birth to 11 years by means of 16 different tests assessing over 200 abilities. Of over 2,200 comparisons between various exposure metrics and neurodevelopmental outcome measures, the number of statistically significant findings was fewer than expected by chance alone. All children were reported to be within normal ranges of performance. Performance of more highly PCB-exposed breast-fed children was not diminished, but was positively correlated with duration of breast-feeding. Some negative correlations reported between neurodevelopment and PCBs were equally attributable to lead or mercury. The findings were generally inconsistent and lacked specificity, credible evidence of a dose-response, or any assurance of a temporally correct relationship. Although the authors reported associations between PCB exposure and neurodevelopmental effects, our analysis found no support for that view. Those purported findings were more likely due to imprecise and inconsistent exposure measurements, poor measures of effect, uncorrected multiple comparisons and other improper statistical methods, and inadequate control for confounders and covariates. Thus, although the human biological plausibility, a causal association between this ascribed cause (PCBs) and this purported effect (impaired neurodevelopment) is without empirical support.

1713

APPLICATION OF QSARs TO PREDICT TRANSFORMATIONS OF POLYHALOGENATED ALIPHATIC COMPOUNDS (PHA) IN HOMOGENEOUS AND HETEROGENEOUS SYSTEMS.


Under anaerobic reducing environments, PHAs may undergo reductive dehalogenation reactions, yielding products that may be less or more toxic than the parent compounds. The toxicity of the parent compounds and their dehalogenated products depend on their concentration in the environment, which in turn depends on
the kinetics of the dehalogenation reaction. Quantitative Structure Activity Relationships (QSARs) were developed to predict rates of transformation of PHAs in nine homogeneous and heterogeneous model systems obtained from literature. The model systems are representative of engineered and natural environments where PHAs are reduced either in the aqueous phase or at the surface of metals, oxides or hydrocarbons. The descriptors for predicting rates depended on the rate-limiting step in the compounds' reactions with reductants. Statistical techniques such as regression, factor and cluster analyses were used to examine rate-limiting steps, which are due to diffusion, sorption or dissociative electron-transfer (DET) in the transformation of PHAs in various studies. Descriptors that were chosen to describe DET were calculated using quantum chemistry, while diffusion and sorption of reductants and products were described using physicochemical properties of the PHAs. Results from QSARs for homogeneous systems indicate that the rate-limiting step is dissociative one-electron transfer. Statistical analysis results indicate that in heterogeneous systems, the transformation of methanes is limited by electron transfer. The results also indicate that transformation of ethenes is limited by mass transfer, while ethynes are limited by both mass and mass electron transfer. Experiments were carried out for two new compounds to verify the QSARs for two different homogeneous systems. The difference between measured and predicted rate constants was within the uncertainty limits at 95% confidence, thereby demonstrating the predictive ability of QSARs for transformation of PHAs in homogeneous systems.

1714 PREVENTION BY CHLOROFORM AND METHIONINE OF DICHLOROACETIC ACID AND TRICHLOROACETIC ACID-INDUCED HYPOXYMATHELYATION OF GENES AND OF THE DICHLOROACETICS PROMOTER OF LIVER AND KIDNEY TUMORS IN MICE.

P. M. Kramer, L. Tao, B. B. Conran, M. Edelbrock and M. A. Preitea. Pathology, Medical College of Ohio, Toledo, OH.

Chloroform, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are mouse liver carcinogens. The effect of chloroform and methionine on DCA and TCA-induced hypoxymethylation of genes was determined. B6C3F1 mice were administered chloroform (0, 400, 800 and 1, 600 mg/kg) in their drinking water and 500 mg/kg DCA or TCA daily by gavage. DCA, TCA and to a much lesser extent chloroform caused hypoxymethylation of the c-myc and IgF-II genes. Co-administration of chloroform prevented only DCA and not TCA-induced hypoxymethylation of the genes. Methionine at 0, 4, 8 and 0, 000 mg/kg diet was administered to B6C3F1 mice with 0. 35 gm/l DCA or 4. 0 gm/l TCA administered in the drinking water. Methionine prevented both DCA and TCA-induced hypoxymethylation of the genes. The relationship of hypoxymethylation of genes to tumor development was tested in female and male B6C3F1 mice initiated on day 15 of age with N-methyl-N-nitrosourea. At 5 weeks of age, the mice started to receive in their drinking water DCA (3. 2 gm/l) or TCA (4. 0 gm/l). The mice also received chloroform at 0, 800 or 1, 600 mg/kg in their drinking water or methionine at 0, 4, 000 and 8, 000 mg/kg in their diet. Chloroform prevented DCA, but not TCA promotion of liver and kidney tumors. In mice, TCA promoted kidney tumors while DCA promoted liver tumors only when co-administered with chloroform. Hence, chloroform prevented only DCA and not TCA hypoxymethylation of the c-myc and IgF-II genes and promotion of liver tumors. Thus the ability to prevent hypoxymethylation of the genes was associated with the ability to prevent tumor promotion supporting the involvement of hypoxymethylation with tumor promotion.

1715 KINETIC AND MECHANISTIC STUDIES ON THE HYDROLYSIS OF HALOGENATED ETHYLENE OXIDES.

T. Yoshioka and P. P. Guengerich. Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN.

Chlorinated ethylenes, e.g. trichloroethylene (TCE) and tetrachloroethylene (PCE) have been shown to be toxic and carcinogenic. P450-catalyzed bioactivation of these compounds to electrophilic ethylene oxides, followed by rearrangements of the oxides to acetylenes, are thought to be related to their toxicities. TCE oxide has been shown to generate adducts with lysine and proteins. In order to elucidate the inherent reactivity of these oxides, comparative kinetic studies on the hydrolysis and rearrangement were performed by using TCE oxide, tribromoacetylenes (TBE) from the surface of TCE oxide, and PCE oxide in a MO/SH/KOH buffer with first-order kinetics. TBE oxide being the most reactive. TCE and TBE oxides gave similar decomposed products of dichloroacetic acid (dibromoacetic acid in the case of TBE oxide), formic acid, glyoxylic acid and carbon monoxide, with different ratios. Dibromooxalic acid was the major product of TBE oxide, consistent with formation of a bromooxazole intermediate to give the acid through an intramolecular rearrangement. PCE oxide yields trichloroacetic acid in a very low yield under the same conditions. In phosphate buffers, the oxide gave oxalyl phosphate and oxalic acid as well as trichloroacetic acid. These results indicate that PCE oxide decomposes through two pathways; one is an intramolecular chloride migration to give trichloroacetic acid and the other is a hydrolysis of the oxide to give oxalyl chloride which is known to react to give the phosphate. The phosphate decomposed gradually to oxalic acid with first-order kinetics. Differences of reactivity in the decomposition reactions of these oxides may affect their reaction with intracellular nucleophile molecules and both the pattern and extent of the adduct formation. (Supported in part by USPHS R01 ES10546 and P30 ES00267)

1716 MASS SPECTROMETRY STUDIES OF OLGONUCLEOTIDES CROSS-LINKED TO ALKYLGLUTAMINE TRANSFERASE VIA ETHYLENE DIBROMIDE.

K. M. Williams, L. Liu, A. E. Pegg and P. P. Guengerich. *Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN* and *Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA.*

O\textsuperscript{6}-Alkylglutamine transferase (AGT) is a DNA repair protein that normally removes alkyl groups from the O\textsuperscript{6} atom of guanine or the O\textsuperscript{4} atom of thymine, with Cys\textsuperscript{145} as the active site for the alkyl group. However, AGT expression leads to increased cytotoxicity and mutagenicity of ethylene dibromide in Escherichia coli cells that lack endogenous AGT activity (Abri et al. Mol. Carcinog. 12, 110-117, 1995; Liu et al. Mutat. Res. 452, 1-10, 2000). Ethylene dibromide or 2-bromoethanol was administered with or without 5-azacytidine to rats. Tissue homogenates were analyzed for the presence of oligonucleotides, and tryptic-digested samples were characterized by matrix-assisted laser desorption ionization (MALDI) and electrospray ionization mass spectrometry (MS). In the absence of oligonucleotides, both ethylene dibromide and 2-bromoethanol react with AGT to form an -SC\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}OH adduct at Cys\textsuperscript{145}; ethylene dibromide apparently forms this adduct via an epissulfonium ion that reacts with water to release the 2-bromoethanol. In the presence of oligonucleotides, the reaction of ethylene dibromide with AGT occurs in a similar manner. In the presence of 2-bromoethanol, thymine and guanine residues in AGT were observed to be modified with the presence of oligonucleotides. The contributions of the G and T modifications to mutagenicity and cell death are under investigation. These findings provide an example of a DNA repair protein acting to increase the genotoxicity of a chemical. (Supported in part by USPHS R35 CA44459, R01 E10546, P30 ES00267, T52 ES07028)

1717 THE INFLUENCE OF ADVANCED AGE ON THE TIME COURSE OF THE HEPATIC TOXICITY OF BROMODICHLOROMETHANE.

A. McDonald, Y. M. Sey and J. E. Simmons. NHEERLIO, USEPA, Research Triangle Park, NC.

Chemical disinfection, by chlorination or by ozonation followed by either chlorine or chloramine, of water containing organic matter results in the production of a variety of halogenated byproducts, which includes trihalomethanes (THMs) such as bromodichloromethane (BDCM). Exposure to BDCM is virtually ubiquitous across all segments of the US population, including the aged. The present study evaluated the influence of advanced age on the time course of BDCM-induced hepatoxocity in young (−90 day old) and aged (24-28 month old) male F-344 rats. Water administered 0.5, 0.5, 1.0 or 2.0 mmol BDCM/kg by gavage in an aqueous vehicle (10% Alkamul as a gavage volume of 5 ml/kg at −3 p.m.). Hepatotoxicity was assessed, in separate experiments, at either 24 hr or 48 hr after dosing, by determining the serum activities of aspartate dehydrogenase (SDH) and alanine (ALT) and asparagine (AST)aminotransferases. The hepatotoxicity of BDCM was dose-dependent in both young and old rats. Based on these serum markers, the hepatotoxic response to BDCM was dependent on age, with greater toxicity observed in the aged rats. At both 24 and 48 hr, the differences between young and aged rats were most apparent at the highest dosage (2.0 mmol/kg). At 2.0 mmol/kg, little or no recovery was apparent between 24 and 48 hrs. These results are in contrast to results previously obtained (Lilly et al., 1997; McDonald et al, 2001) with chlorofom, another THM ubiquitous in chlorinated or chloraminated water, where recovery was apparent in both young and aged rats between 24 and 48 hr and consistent with BDCM results in young rats (Lilly et al., 1997). In conclusion, these data suggest that advanced age alters the hepatic response to BDCM, indicating that the aged may be a susceptible subpopulation for TTHMs. (Abstract may not reflect EPA policy.)
1718 SENSITIVE METHODS FOR THE DETECTION OF TRACE LEVELS OF TRICHLOROETHYLENE (TCE) IN RAT PLASMA.
A. M. Dixon, S. D. Brown, S. M. Muradlibas, L. V. Bruckner and M. G. Barlow. Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA.

TCE is a widely used solvent and metal degreasing agent that is frequently found in US drinking water supplies in trace levels (i.e., a few ppb to ppt). Current cancer and noncancer risk assessments of the chemical predict that exposure to even minute amounts of TCE may be associated with risks to humans. Due to a lack of analytical sensitivity, it has not yet been possible to conduct experiments to evaluate the validity of some key assumptions in the risk assessments. Two analytical methods were developed for this purpose and compared to one another. The first is a more traditional gas chromatography (GC) liquid-liquid extraction (LLE) method, while the second uses the relatively new technology of solid phase microextraction (SPME). With SPME, a fiber is inserted into the headspace of a sample vial to allow TCE to adsorb onto the fiber. The fiber is then placed into the injection liner of the GC where TCE is desorbed. SPME has the advantage of simpler, more rapid sample preparation. High resolution GC/MS was used for the analysis of plasma samples by both methods. For each technique, 100 ul of plasma were spiked with 100 ul of an aqueous TCE solution (1-10, 000 ng/ml). With LLE, 100-200 ul of diethyl ether were added to each sample. These samples were then centrifuged before analysis. Samples run on SPME were transferred to vials where the SPME fiber was inserted for 1 minute, then removed and inserted into the GC for analysis. The limit of detection (LOD) was found to be 1 ng/ml (ppb) for both methods. This is an improvement over the LOD of 25 ng/ml for the GC headspace method currently in use in our laboratory. With the increased analytical sensitivity, trace level studies involving first-pass metabolism of TCE and interaction of TCE with inducers/inhibitors of metabolism are now possible. (Supported in part by DOE Grant # DEFC02 98CH10902 and ATSDR contract # 68164).

1719 PRESENCE AND ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR IN NEURONAL VASCULAR ENDOTHELION.
C. E. Ilkhanot and T. A. Gasiorkw. Environmental Medicine, University of Rochester, Rochester, NY.

The aryl hydrocarbon receptor (AHR) is a member of the bHLH-PAS family of transcriptional regulatory proteins known functionally as a modulator of xenobiotic toxicity. Many of the mechanisms involved in AHR activation and the transcriptional events following ligand binding have been well characterized, but not all tissue-specific targets or functional consequences have been defined. Although the brain is a target for AHR mediated toxicity, consequences localized to regions, such as the cerebral vascular endothelion are unknown. Receptor activation and subsequent CYP1A1 upregulation could compromise integrity and function of the neuronal endothelion and therefore, the blood-brain barrier. The purpose of these studies was to examine the presence and activity of AHR in brain endothelion. Receptor mRNA and protein were found regionally throughout the brain of adult C57BL/6 mice. Relative quantitation by real-time RT-PCR indicated receptor mRNA within the cerebellum > cortex > striatum > thalamus and hypothalamus. Furthermore, the receptor was also detected in total RNA and protein isolated from primary cortical endothelion cultures. Although its abundance is nearly ten-fold less in the whole cortex and endothelial cell culture than in liver, exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), induced upregulation of CYP1A1 transcription. We hypothesize that receptor activation and downstream gene transcription upregulation in these cells may have functional consequences in terms of oxidative stress and barrier permeability. If these consequences occur in vivo, they could play a significant role in the potentiation of vascular injury that may underlie the development of various neurodegenerative diseases. (Supported by NIH grant ES09430, Training Grant ES07926, and Center Grant ES1247).

1720 KERATINOCYTE INTERLEUKIN-1B EXPRESSION IS MEDIATED BY THE ARYL HYDROCARBON RECEPTOR.
D. V. Henley, C. J. Bellone, D. A. Williams and M. E. Rub. 'Pharm/Phy Science, Saint Louis University, St. Louis, MO and 'Molec Micro & Immunology, Saint Louis University, St. Louis, MO.

Halogenated aromatic hydrocarbons represent a wide variety of environmental contaminants. Human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most potent member of the halogenated aromatic hydrocarbon family, produces several adverse health effects, including chloracne, a skin rash characterized by epidermal hyperkeratinization and inflammation. The proinflammatory cytokine interleukin-1β (IL-1B) has previously been suggested to play a role in the pathology of chloracne. TCDD elicits its effects by binding to the aryl hydrocarbon receptor (AhR) and forming an AhR heterodimeric nuclear complex. To test the hypothesis that the IL-1B gene is regulated by an AhR-mediated mechanism, differentiating SCC-12 and HaCaT human keratinocytes were treated with TCDD or vehicle. Western blot analyses were performed to determine the effect of TCDD on IL-1β protein expression in these keratinocytes. Time and dose dependent increases in prointerleukin-1β (pIL-1β) were seen in both SCC-12 and HaCaT cells following TCDD treatment when compared to vehicle controls. Northern blot analyses were performed to ascertain the effect of TCDD on IL-1β steady state mRNA levels. TCDD treatment resulted in dose and time dependent increases in IL-1β mRNA in both SCC-12 and HaCaT cells when compared to vehicle controls. Increases in CYP1A1 mRNA, a gene known to be transcriptionally activated by TCDD in cells undergoing terminal differentiation, paralleled increases in IL-1β mRNA with respect to time in both cell lines. Both cell lines were treated with TCDD in the presence and absence of α-naphthoflavone, an AhR antagonist, to determine if the TCDD-mediated increases in IL-1β protein and steady state mRNA were AhR-dependent. Western and Northern blots revealed that the AhR antagonist attenuated TCDD-mediated increases in IL-1β. These results confirm that the AhR regulates TCDD-mediated increases in IL-1β gene expression in differentiating keratinocytes. (Supported, in part, by NIH ES05968).

1721 DIOXIN TOXICITY: LARGE-SCALE GENE EXPRESSION IN A RESISTANT RAT MODEL.
M. A. Franc, R. Pohjanvirta, J. Tuominen and A. B. Oke. 'Dept of Pharmacology, Univ of Toronto, Toronto, ON, Canada, 'Veterinary Medicine, Univ of Helsinki, Helsinki, Finland and 'National Public Health Institute, Kuopio, Finland.

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) is exceptionally toxic and also alters expression of a wide spectrum of genes in cell culture and in vivo via the Ah receptor (AhR). Dysregulated gene expression probably triggers toxicity but the dysregulated genes that are key to lethality and other toxicities remain undefined. We are using gene expression arrays to identify and compare TCDD-regulated genes in rat strains that differ dramatically in their sensitivity to TCDD lethality and in their AhR structure: Long Evans Turko AB (LE) [LD₅₀ ~ 10 μg/kg] and Han/Wistar Kyoto (H/W) [LD₅₀ > 9600 μg/kg]. Our postulate is that genes whose expression is altered by TCDD in sensitive LE rats but not in resistant H/W rats are the genes most likely to be relevant to lethality. Rats were given a single TCDD dose of 50 μg/kg intragastrically. Liver was removed after 24 hours and RNA was reverse-transcribed and labeled with 32P prior to hybridization to Clontech Atlas 1.2 rat arrays. Of 1185 genes on the array, TCDD caused 65% (67 genes) to change in expression (1.5-fold, up or down) in the LE rat and 8% (90 genes) in the H/W. Of these, only 33 genes were common to both strains. Genes well-evaluated as being responsive to dioxins (CYP1A1, CYP1A2, CYP1B1, GST-Y) were upregulated in both LE and H/W strains, establishing the validity of this array for detection of responses to TCDD. In addition to altered expression of these "positive control" genes, the experiments revealed altered expression of several novel genes. Many of these are currently reported to be induced by TCDD in rat strains. In addition, as more genes are being studied with the Clontech array and with a glass microarray from the Ontario Cancer Institute (>15,000 unique cDNA sequences) to establish a hierarchy of genes whose TCDD-responsiveness consistently differs between dioxin-sensitive versus dioxin-resistant rats to identify candidate genes that are most likely to be involved in dioxin lethality.

1722 LOW IN VITRO HEMATOMATOIC TOXICITY OF TENOFUVIR RELATIVE TO OTHER NRTIs: COMPARISON OF RAPID THROUGHPUT CELISA AND COLONY ASSAY DATA.

Nucleoside reverse transcriptase inhibitor (NRTI)-containing regimens have been demonstrated to produce various hematological disorders. In comparison with untreated control groups, anemia and/or neutropenia have been detected at a higher rate in HIV patients treated with ZDV (Richman et al., 1987), d4T (Bowen et al., 1993), or ddC (Yarchun et al., 1988). Tenofuvir is an acyclic nucleoside analog with potent in vitro and in vivo antiretroviral activity. Its lipophilic produrg, tenofovir disoproxil fumarate, is currently in late stage clinical development as a novel oral agent for treatment of HIV infection. The present study was designed to help define the in vitro toxicity profile of tenofovir and other NRTIs by assessing their cytotoxic effects in human hematopoietic progenitor cells. Two different in vitro toxicity assays were used: the clonogenic "colony assay" and the 96-well filter plate-based CELISA. Human: CD34+ progenitor cells were isolated from two individual
donors, treated with lineage-specific growth factors to induce myeloid and erythroblast differentiation and exposed to NRTIs for 10 days. Lineage-specific differentiation was determined by use of the CELISA platform technology utilizing lineagespecific fluorescent antibody-based immunoassays and colony counting assays. The tested NRTIs displayed a range of toxicities with IC₅₀ values ranging from >200μM to <60μM. The relative toxicities of the NRTIs were ddC > ZDV > d4T > 3TC = tenofovir. ZDV and d4T had a 10-fold increased toxicity in one donor relative to the second and exhibited marked lineage-specific toxicities, the erythroid lineage being more sensitive in each case. The CELISA data correlated with colony assay data in all cases. We conclude that lead optimization of NRTIs and other drugs can be greatly accelerated through use of the rapid-throughput CELISA assay of in vitro cell differentiation.

1723
THE ROLE OF REACTIVE OXYGEN SPECIES IN MEDIATING THE ACTIVITY OF THE C-MYB ONCOGENE.
L. M. Wino1 and S. A. Noca2, Pharmacology and Toxicology, Queens University, Kingston, ON, Canada; and Molecular Genetics and Microbiology, University of New Mexico Health Science Center, Albuquerque, NM.

The incidence of childhood leukemias has increased 20% over the last two decades. Exposure to several drugs and environmental toxicants has been proposed to be a primary cause for this increase. Mutated or overexpressed versions of the c-myc oncogene are believed to play a pivotal role in the development of a variety of leukemias and other tumors. The c-myc gene encodes a highly conserved 78 kDa transcription factor that has been implicated in a diverse set of biological processes, including the regulation of cell proliferation, differentiation, and apoptosis. While the structure of the c-Myc protein and the regulation of c-myc gene expression have been studied in great detail, little is known about the upstream activators that regulate c-myc protein activity. Furthermore, nothing is known about the effects of xenobiotic exposure on c-myc activity. The toxicity of many xenobiotics is mediated by xenobiotic bioactivation to a reactive free radical intermediate, which is not detoxified may initiate the formation of reactive oxygen species (ROS). We hypothesize that exposure to xenobiotics that lead to increased ROS mediate leukemogenesis by disrupting crucial cell signalling pathways involving the c-myc oncogene. To evaluate this hypothesis, quail QT6 cells were transfected with a Myc-responsive luciferase reporter plasmid derived from the chicken mmt-1 promoter, along with control vector DNA or a plasmid expressing c-Myc. The following day cells were serum starved for 24 hr and then exposed to 400 μM H₂O₂ for 5 minutes. Luciferase activities were then measured in cell lysates to evaluate c-Myc activity. Results of these studies showed that cells exposed to H₂O₂ had significantly increased c-Myc activity compared to cells exposed to the vehicle control and support the hypothesis that ROS mediate the transcriptional activity of this oncogene. The hypothesis that xenobiotic-induced ROS production leads to aberrant c-Myc signalling is currently being investigated.

1724
SIMULTANEOUS DETERMINATION OF SIX URINARY PORPHYRINS IN THE UNTREATED BEAGLE DOG USING LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY.
T. P. O'Neill1, W. Bu1, C. P. Chegeli1, N. Myers1, J. D. McCarty2, P. L. Stenson1 and D. Sved1, Toxicology, WIL Research Laboratories, Airdland, OH and 1Toxicology, FMC Corporation, Princeton, NJ.

Porphyria is converted to heme by a series of enzymatic steps. Deficiencies in these enzymes leads to porphyria, which may have specific toxicological consequences. Evaluation of porphyrins in urine can be useful in the recognition of these toxicological sequelae. A liquid chromatograph-tandem mass spectrometric (HPLC-MS-MS) method without sample pretreatment was developed and validated for determination of urinary porphyrins. The acidified urine sample was directly injected for HPLC/MS/MS analysis using a reverse-phase column with gradient elution, and ES positive ion detection and quantitation. The mass spectrometer was operated in the multi-reaction monitoring (MRM) mode and six porphyrins (mesoporphyrin, coproporphyrin, 5-carboxyporphyrin, 6-carboxyporphyrin, 7-carboxyporphyrin and uroporphyrin) were detected with excellent sensitivity and selectivity. Values obtained in the untreated beagle dog corresponded to not present, 0.1361, 0.0196, below lower limit of quantification, 0.0328 and 0.0624 nmol/mL, respectively, which are within expected normal values. The lower limit of quantitation was 0.014 nmol/mL for each porphyrin, and a good linear response for calibration standards ranging from 0.01 to 1.0 nmol/mL for each porphyrin was demonstrated. This method can be used for determining the effects of chemicals and pharmaceuticals on urinary porphyrin excretion profiles in preclinical animal studies and in the investigation or diagnosis of porphyria in clinical studies.

IN VIVO DISTRIBUTION AND TOXICITY OF THE NOVEL COPPER-BASED ANTI-CANCER COMPOUND: CASIOPEINA II.
A. E. Viscaya-Ruiz1, A. Rivero-Muller1, J. James1, L. Ruiz-Ramirez2 and M. Dobrza1, SBS, University of Surrey, Guildford, United Kingdom; 1Facultad de Quimica, UNAM, Mexico, D.F.; 2Toxicology, CINVESTAV-IPN, Mexico, D.F. Sponsor: M. Cobian.

The in vivo fate and toxicity of the novel copper-based anticaner agent, casiopeina II [Cu(4, 7-dimethyl-1, 10-phenanthroline/glycine) NO₃], was studied. Casiopeinas are a family of squaraine planar copper-coordinated complexes that show promising anticaner activity. The phenanthroline ligand of CuII tris(3, 3'-diaminobenzaldehyde) was labeled to follow the rapid-throughput of the two components of the complex. After a single iv administration of the casiopeina II (CII) (5mg/kg dose) in the rat the complex was rapidly distributed to tissues (liver and kidney), the 1H-phenanthroline was excerted via urine and feces while the copper appeared to be predominantly reabsorbed with a small amount being excreted via feces. Differences in tissue copper retention and 1H-phenanthroline elimination suggest an in vivo dissociation of the complex. The major toxic effect attributed to CII administration was an hematocrit anemia (reduced hemoglobin concentration, red blood cell count and packed cell volume as well as leukocytosis and neutrophilia) observed 12 hours and 5 days after administration, attributed to a direct erythrocyte oxidative damage. Increased reticuloocyte levels and presence of normoblasts in peripheral blood 5 days post-administration indicated an effective erythropoietic response and tendency towards recovery at 15 days. Increase in spleen weight was attributed to an increased uptake of damaged erythrocytes by the reticuloendothelial system. Erythropoietin pretreatment before CII administration reduced the hematological effects. In conclusion, data from copper and 1H distribution and excretion suggests that CII complex is readily dissociated in vivo, which could give rise to the acute toxic effect in erythrocytes due to the generation of free radicals and oxidative stress from the reduction of Cu(II) to Cu(I). Improving the stability of the complex in vivo could increase its biological activity and thus its anticancer potential.

1726
SINUSOIDAL ENDOTHELIAL CELL INJURY DURING HEPATOTOXICITY FROM MONOCROTONALINE AND BACTERIAL LIPOPOLYSACCHARIDE COEXPOSURE.
S. B. Yue, B. L. Copple, R. E. Ganey and R. A. Roth, Pharmacology and Toxicology, National Food Safety and Toxicology Center, and the Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.

A previous study demonstrated that a nonoxo dose of bacterial lipopolysaccharide (LPS) given 4 hours after a small dose of monocrotonaline (MCT) resulted in synergistic hepatotoxicity within 12 to 18 hours after MCT administration. Histopathologic analysis revealed centrilobular and midzonal lesions characteristic of those associated with larger toxic doses of MCT and LPS, respectively. Moreover, within these lesions there was pronounced hemorrhage, loss of sinusoidal architecture, and the loss of central vein integrity, suggesting a vascular component to the injury. It has been postulated that injury to sinusoidal endothelial cells (SECs) creates microcirculatory disturbances that contribute to hepatic parenchymal cell injury. To begin to explore this hypothesis, the association of SEC injury with hepatic parenchymal cell injury was evaluated. 4 hours, a significant increase in plasma hyaluronic acid (HAI), a biomarker of SEC injury, was observed in animals coexposed with MCT/LPS. This increase in SEC injury was confirmed through immunohistochemistry with a rat endothelial cell antibody (RECA-1). Morphometric analysis revealed a decrease in RECA-1 staining intensity (ie., a loss of SECs) in livers of rats treated with MCT/LPS. The decreased staining occurred in both centrilobular and midzonal regions of the liver lobule. A significant increase in liver hemoglobin, a biomarker of hemorrhage, was also observed at 18 hours in coexposed animals, further suggesting circulatory and vascular disturbances. Finally, SEC injury developed before the onset of hemorrhage and parenchymal cell injury, an observation consistent with the hypothesis that SEC injury plays a causal role in this model of liver injury. Supported by NIH Grant ES 04139.

1727
NAPROXEN SODIUM NEPHROTOXICITY: INTERSPECIES DIFFERENCES.
P. B. Sensere1, D. A. Baron1, K. M. Stanfeld1 and K. N. Khan1, 1Global Toxicology, Pharmacia, Stokie, IL and 1Toxicology, Tokeida, Linclonshire, IL. Sponsor: M. Schlosser.

Cyclooxygenase (COX) exists in two isoforms, COX-1 and COX-2. Constitutive COX-2 is found in the mucosal densely (MD) and thick ascending limb of the loop of Henle in rodents and dogs, and is absent at these sites in normal adult humans and monkeys. This difference in COX expression may be responsible for the contrasting nephrotoxicity profiles of NSAIDs in these species. We compared the toxicity profile of naproxen sodium (a non-selective COX inhibitor) in dogs and monkeys.
Beagle dogs and moyamoya monkeys were given nanoparticle sodium orally for six weeks at dosages up to 10 and 150 mg/kg/day. The dogs exhibited a 5-fold increase in BUN and a 1:1-fold increase in creatinine suggesting pre-renal azotemia. Kidneys exhibited tubular atrophy and fibrosis in the outer cortex of 4/6 dogs and renal papillary necrosis in 3/6 dogs. The lesions correlated with increases in BUN. In contrast, monkeys exhibited a less pronounced increase in BUN (2-fold) and did not develop renal papillary necrosis or cortical tubular atrophy and fibrosis; however, obstructive nephropathy, presumably due to drug accumulation in the renal tubules, was observed. Monkeys also exhibited an increase in creatinine (3.6-fold) which was likely secondary to the obstructive nephropathy. These data suggest that dogs are more sensitive to NSAIQ-related nephrotoxicity in comparison to monkeys. The results in dogs appear to be secondary to regional hypoxia/ischemia and implicate an effect of nanoparticle sodium on renal hemodynamics. The MD plays an important role in renin angiotensin system; thus, co-localization of high levels of COX-2 at this site in dogs suggest an important role of COX-2-mediated prosstaglandins in normal renal physiology in dogs. The role of COX-2 on renal hemodynamics in the normal kidney appears to be less significant. This study demonstrates that a differential renal response to NSAIQ administration among species can be explained by the interspecies variation of renal COX-2 expression and its species specific role in normal kidney function.

**1728**

**NEUTROPHIL APOPTOSIS AND THE RESOLUTION OF PULMONARY INFLAMMATION.**

**A. L. Wieghoff, K. L. Reed, T. R. Webb and D. B. Warheit. DuPont Hawkell Laboratory for Health and Environmental Sciences, Newark, DE.**

The apoptosis, or programmed cell death, of neutrophils and their subsequent engulfment by macrophages is important in the resolution of pulmonary inflammation. Previous studies showed a correlation between inflammation and apoptosis in silica-exposed rats. However, in titanium dioxide (TiO2) exposed rats, only a slight apoptotic potential was seen at very high doses in the presence of measurable levels of inflammation. The goal of this work appears to be to further investigate the relationship to the inflammatory process due to amorphous and crystalline silica. LPS and TiO2 exposure. Rats were instilled with TiO2, Zeeoff 80 (amorphous) or Min-U-Sil (crystalline silica) at both 1 and 5 mg/kg. A group of rats were also instilled with 6 mg LPS. Bronchoalveolar lavage fluid and tissue were collected at 12, 24, 48 and 168 hours after installation. Only the amorphous silica instilled animals displayed measurable apoptosis (detected by ELISA) at 1 mg/kg with the highest level at 24 hours post installation. At 5 mg/kg, apoptosis was highest at 12 hours for amorphous silica, and at 24 hours for the other substances tested. By 168 hours, only crystalline silica instilled animals had any measurable response. Pulmonary inflammation, as evidenced by neutrophil influx correlated with the apoptotic measurements. DNA array technology was employed on lung tissue from sham, Zeeoff 80, Min-U-Sil and LPS animals and expression of several genes related to apoptosis were upregulated. These studies confirmed that TiO2 exposure causes low levels of inflammation and apoptosis. Amorphous silica and LPS produces a strong, but transient inflammatory response while instillation of crystalline silica results in sustained pulmonary inflammation and measurable apoptotic tissues after a week.

**1729**

**COMPARISON OF LOW DOSES OF AGED AND FRESHLY FRACTURED SILICA ON PULMONARY INFLAMMATION AND DAMAGE IN THE RAT.**

**D. W. Porter, M. Barger, V. A. Robinson, S. Leonard, D. Landsittel and V. Casanova. HELED, NIOSH, Morgantown, WV.**

Most previous studies of silica toxicity have used relatively high doses of silica. In this study, rats received by intratracheal installation either vehicle, aged or freshly fractured silica at a dose of either 5 mg per rat once a week for 12 weeks (total dose of 60 mg) or 20 mg per rat once a week for 12 weeks (total dose of 240 mg). One week after the last exposure, bronchoalveolar lavage (BAL) was conducted and pulmonary inflammation, macrophage activation (AM) and pulmonary damage were examined. Specifically, pulmonary inflammation was assessed by BAL polymorphonuclear leukocyte (PMN) yields, AM chemiluminescence was measured as an indicator of AM activation, and first acellular BAL fluid lactate dehydrogenase activity and serum albumin concentration were determined as markers of pulmonary damage. For rats exposed to a total of 60 mg silica, both aged and freshly fractured silica increased PMN yield and AM chemiluminescence above control to a similar degree, but no evidence of pulmonary damage was detected. For rats exposed to a total of 240 mg silica, aged or freshly fractured silica increased PMN yield and AM chemiluminescence above control. However, total and NO-dependent AM chemiluminescence was greater for rats exposed to freshly fractured silica compared to aged silica. Exposure to 240 mg aged or freshly fractured silica also resulted in pulmonary damage, but the extent of this damage did not differ between the two types of silica. The results suggest that exposure to silica levels far lower than those previously examined can cause pulmonary inflammation and damage. In addition, exposure to freshly fractured silica causes greater generation of reactive oxygen and nitrogen species from AMs (measured as total and NO-dependent AM chemiluminescence) in comparison to aged silica, but there is an apparent threshold below which this difference does not occur.

**1730**

**INCREASED IL-6 AND IL-8 EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS EXPOSED TO CARBON ULTRAFINE PARTICLES.**

**R. Silbajoris 1, A. G. Lenz 1, J. Jasper 1 and J. M. Samet. 1, NHEERL, ORD, USEPA, Research Triangle Park, NC, 2, DGIST-Institute for Inhalation Biology, Neuberg, Germany, 3, CELMRL, UNC-CH, Chapel Hill, NC.**

Ultrfine particles are a potentially toxic constituent of particulate matter (PM) and may contribute to the health effects of ambient PM. Exposure to extracts or components of PM has been shown to evoke inflammatory responses in cultured normal human airway epithelial cells (NHBE). One of these responses is the expression of pro-inflammatory proteins such as IL-6 and IL-8. The aim of the present study was to characterize the effect of these particles on IL-6 and IL-8 expression in NHBE. Particles were generated by spark discharge and had a diameter of 75 nm and a surface area of 750 m2/g. Cells were exposed to 0 to 66 μg/ml of EC for 2 to 30 hr and levels of IL-6 and IL-8 mRNA and protein were measured using real-time fluorescent PCR and ELISA, respectively. After 2 hr of exposure to 33 or 66 μg/ml EC showed 9-fold and 12-fold increases in IL-6 and IL-8 mRNA, respectively. Doubling the amount of EC to 66 μg/ml resulted in a 10-fold increase in IL-6 mRNA and a 20-fold increase in IL-8 mRNA. To minimize underestimation of IL-6 and IL-8 protein release caused by non-specific protein binding to EC, a pulsed exposure protocol was used. After exposing NHBE to media with 33 or 66 μg/ml EC for 8 hr to initiate mRNA induction, media was changed so that protein release would take place in media without EC. When compared to controls, this regimen resulted in 5-fold elevations in IL-6 and IL-8 release by NHBE that were exposed to EC for 24 hr. These data show that carbon ultrate fine particles not only induce inflammatory cytokines and chemokines in vitro, but also contribute to systemic effects of ambient PM inhalation. Further studies will be required to identify the mechanism of IL-8 expression induced by exposure to EC. This abstract of a proposed presentation does not necessarily reflect EPA policy.

**1731**

**RESPONSIVENESS OF ALVEOLAR MACROPHAGES FROM INOS KNOCKOUT OR WILD TYPE MICE TO AN IN VITRO DMS OR SILICA EXPOSURE.**

**P. C. Zedler, V. A. Robinson and V. Casanova. NIOSH, Morgantown, WV.**

The role of nitric oxide (NO) in pulmonary disease has been controversial with both anti-inflammatory (sequestrating radicals and inhibiting NF-kB activation) and pro-inflammatory (forming highly reactive nitrogen species and augmenting NF-kB activity) actions reported. Therefore, a project has been initiated to determine whether deletion of the INOS gene in the C57BL/6 mouse alters the pulmonary response to lippopolysaccharide (LPS) or silica. The objective of the initial phase of this study was to determine the responsiveness of alveolar macrophages (AM), harvested from naive wild type mice (WT) and INOS knockout (KO) mice, to in vitro LPS or INOS knock-out primary AM. Primary AMs were obtained by bronchoalveolar lavage from age and weight matched INOS KO and WT mice. The cells were treated with INFγ-Y (50 U/ml), INFγ-Y (50 U/ml) plus LPS (1μg/ml), LPS (0.01 -100 μg/ml), or silica (25 - 250 μg/ml). Inflammatory parameters included: NO, tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-2 (MIP-2) production, and intracellular generation of hydrogen peroxide and superoxide. Data show a significant increase in NO production upon exposure to INFγ-Y +/- LPS in the WT but not INOS KO AM's. NO production by KO or WT AM's was not increased by in vitro exposure to LPS or silica alone. LPS, but not silica, induced TNF-α and MIP-2 production with the KO AM's being more responsive than WT. Basal intracellular production of hydrogen peroxide and superoxide was significantly greater in KO compared to WT AM's. In addition, LPS (10 μg/ml) or silica (100 μg/ml) stimulated intracellular production of hydrogen peroxide was lower in KO AM's. In conclusion, when compared to WT AM's, INOS KO AM's exhibit a decreased ability to generate reactive oxygen species in response to LPS or silica. However, INOS KO AM's exhibit enhanced inflammatory cytokine and chemokine production in response to LPS.
was to develop a system that allows for the generation of primary combustion particles followed by controlled dilution and conditioning, and subsequent whole-animal inhalation exposure. In these pilot studies a heavy oil #5 was burned in a residual oil combustor previously shown to produce approximately 150 mg/m^3 of PM. The particles were then passed through a 2.2 μm cyclone and diluted with clean ambient air. The resulting PM was then directed to a Hamann animal exposure chamber equipped for continuous monitoring of CO, CO2, NOx, SO2, as well as total particle concentrations. Mean PM 2.5 concentrations ranged between 2 and 5 mg/m^3 with 40-50 ppm SO2 and 20-30 ppm NOx. Balb/C mice were exposed to the combustion atmosphere for 4 hrs and immediately and 24 hr later assessed for acute lung injury (both strains) and susceptibility to Streptococcus zooepidemicus infection (CD1 strain). Exposure to the emission atmosphere caused a significant increase in neutrophils and lactate dehydrogenase (LDH) in bronchial lavage fluid (BAL) of both strains of mice but did not affect levels of protein. Mortality to infection was low in all animals and was not affected by the emission exposure. We conclude that this acute exposure caused mild pulmonary injury and inflammation in a similar fashion to other oxidant pollutants but did not affect host defenses to streptococcal infection. Future studies will determine the relative contribution of gas phase particles on these inflammatory responses, and will test whether animals with other forms of cardio-pulmonary disease are further compromised by such exposures. This abstract does not necessarily reflect EPA policy.

1733 COMPARISON OF SINGLE AND MULTIPLE EXPOSURES OF CONCENTRATED AIR PARTICLE (CAP) PATHOPHYSIOLOGIC RESPONSES IN HEALTHY RATS.

Several studies have reported that exposure to CAPs from various cities causes pulmonary inflammation in rats. The purpose of this study was to determine whether CAPs from the Research Triangle Park area could cause similar effects, and to compare responses between single and multiple CAPS exposures. Using a 4 stage Harvard concentrator system which concentrated particles 80-fold, male Sprague-Dawley rats were exposed to CAPs for 4 hours, 3 times a week for 5 weeks. During the last 2 exposures (chamber concentrations of 0.57 and 1.7 mg/m^3 respectively), additional groups of animals were exposed to CAPS or clean air and their acute pulmonary inflammatory responses (18 hr post-exposure) were compared to animals which had been exposed for the entire 5 weeks period. After 2 and 4 weeks of exposure, animals monitored with a Buxco plethysmograph system showed a small exposure-related delayed in enhanced Passive Volumes, suggesting an alteration in ventilatory function. At necropsy, no changes were apparent in bronchoalveolar lavage levels of protein and LDH. The higher CAPS concentration however caused an increase in pulmonary alveolar macrophages and neutrophils. This effect seemed to be driven by the last exposure as animals exposed 14 times to CAPS prior to this exposure did not have significantly greater responses. Further exposure to the lower concentration had no effect indicating either: a Dose-Response effect, or changes in the physicochemical makeup of the PM over the last 2 exposures. Other significant differences included a decrease in lung, ascobate levels following acute exposure to the higher CAPS concentration, and an acute plasma fibrinogen levels. The data show that acute CAPS exposure causes a dose-dependent pulmonary infiltration of cells, depletion of antioxidants, and increased potential for systemic cytokine. These effects did not appear to be worsened with multiple exposures. This abstract does not reflect EPA policy.

1734 PARTICULATE 13-3-B-GLUCAN IS THE MORE POTENT FORM FOR INDUCING PULMONARY INFLAMMATION IN RATS.
S. Young, V. A. Robinson, M. Barger, D. G. Frazer and V. Castranova. Health Effect Laboratory Division, NIOSH, Morgantown, WV.

13-3-B-Glucans, derived from the inner cell wall of yeasts and fungi, are commonly found in indoor air dust samples and have been implicated in indoor dust toxic syndromes. In a previous study, we reported that 1-3,3-B-glucan (zymosan A) induced acute pulmonary inflammation in rats. The present study investigates which form of 1-3,3-B-glucans, particulate or soluble, is more potent in inducing pulmonary inflammation. Zymosan A was suspended with 0.25 N NaOH for 30 min, neutralized, dialyzed for 2 days against deionized water, and particulate and soluble samples were collected. Male Sprague-Dawley rats were exposed via intranasal instillation to NaOH-soluble or NaOH-insoluble zymosan A (1.8 mg/ml, 0.26 ml). At 18 hrs post-exposure, various indicators of pulmonary response were monitored, including indicators of lung damage, such as serum albumin concentration and lactate dehydrogenase activity in acellular bronchoalveolar lavage fluid. Inflammation was characterized by an increase in lavageable polymorphonuclear leukocytes (PMN), pulmonary irritation (breathing frequency increase) and oxidant production (nicotie oxide and chemiluminescence (CL)). Exposure to the particulate fraction of NaOH-reated zymosan caused a significant increase in all these indicators. In contrast, rats exposed to the NaOH-soluble fraction did not show significant increase for most of these indicators except for albumin, PMN and CL. However, these increases were significantly smaller than with exposure to NaOH-insoluble zymosan. Therefore, the results demonstrate that particulate zymosan A is more potent in inducing pulmonary inflammation and damage in rats than the soluble form of this B-glucan.

1735 VANADIUM-INDUCED PULMONARY INFLAMMATION AND APOTOPSIS IN MICE.
L. Wang, R. Medan, C. Huang, X. Shi, R. R. Mercer, L. Miliechicha, V. Castranova and Y. Rojasenskali. PPRB, NIOSH, Morgantown, WV, School of Pharmacy, West Virginia University, Morgantown, WV and School of Medicine, New York University, New York city, NY.

Pulmonary exposure to vanadium and vanadium-containing compounds is associated with acute pulmonary inflammation, characterized by a rapid influx of polymorphonuclear neutrophils with a peak response at 6 h and resolution by 3 d. Sodium metavanadate (V VI) can induce cell apoptosis in vitro, but little is known about relationship of V (VI) and lung cell apoptosis in vivo. We hypothesized that vanadium may induce lung cell apoptosis through reactive oxygen species (ROS) in vivo and neutrophil apoptosis is involved in the resolution of vanadium-induced lung injury. To test this hypothesis, mice were treated with V (V) or saline control, and the bronchoalveolar lavage (BAL) cells were examined at various times for short-lived free radicals by electron spin resonance (ESR) and for apoptosis using terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL). Control mouse BAL cells showed no significant free radical activity and only oxidant alveolar macrophages in the BAL fluids with no evidence of apoptosis. In contrast, V (V) induced a greater level of free radicals and caused apoptosis of BAL cells which were predominantly neutrophils. Catalase blocked both free radical generation and apoptosis induced by V (V). The number of apoptotic cells gradually increased and reached a maximal level by 24 h where it subsequently declined. After 24 h when the vanadium-induced lung inflammation was in the resolution phase, we observed an increased influx of macrophages and their engulfment of apoptotic bodies in the BAL fluid. At 72 h when the total number of neutrophils fell to the baseline level, remnants of apoptotic bodies could still be seen in the cytoplasm of macrophages. We conclude that: (1) ROS, like HO2-, are one of causes of apoptosis induced by vanadium; (2) apoptosis of neutrophils and clearance by macrophages is an important mechanism in the resolution of vanadium-induced lung inflammation.

1736 SYMPATHETIC NERVOUS SYSTEM PLAYS A MAJOR ROLE IN ACUTE COLD/RESTRAINT STRESS INHIBITION OF HOST RESISTANCE TO LISTERIA MONOCYTOGENES.
L. Cao, N. M. Filipsey and D. A. Lawrence. Toxicology, Wadsworth Center, Albany, NY.

Multiple studies have demonstrated that both glucocorticoids and norepinephrine (NE) can modulate immune responses in vivo and in vitro, and it is well known that the sympathetic nervous system innervates both primary and secondary lymphoid organs. Here, we show that acute cold/restraint stress (ACS) significantly lowers host resistance to Listeria monocytogenes (LM) in BALB/c mice, and the involvement of stress hormones corticosterone (CORT) and NE is evaluated. CORT and NE were measured by pretreating mice with the CORT synthesis inhibitor metyrapone and the chemical sympathomimetic drug 6-hydroxydopamine (6-OHDA), respectively. LM burdens were determined 3 days post-infection. 6-OHDA significantly decreased the LM burden in both control and stressed animals. 6-OHDA also completely blocked the stress effects observed in spleens while only partially affecting the liver. The 6-OHDA-uptake inhibitor desipramine confirmed that peripheral sympathoadrenergic nerves and NE depletion and not the nonspecific 6-OHDA toxicity were responsible for the enhanced host defense. In contrast, metyrapone-treated animals had further decreased host resistance to LM after ACS. The results suggest that the peripheral sympathovasculature system (SNS) postganglionic neurotransmitter NE plays a major role in LM host resistance, but there are significant tissue-dependent effects after ACS, while CORT provides a potential protective effect after ACS. Altogether, stress hormones play important roles in stress-modulated host resistance and NE is a major hormone involved in ACS-induced suppression of host resistance in the spleen but not the liver. (Supported by NYSDOH and NIEHS ES15-35606A).
Specific PBR ligands have been used to visualize brain tumors and inflammation using non-invasive imaging techniques such as positron emission tomography. To further develop the use of PBR as a non-invasive marker of inflammation, we used LPS as a model of inflammation in mice. Mice were injected with 0.1, 10, or 30ng/kg LPS. Two days later, mice were injected with the PBR-specific radioligand 

125I-(R)-PK11195. Static planar images revealed increased radioactivity in the brain of both 10 and 30ng/kg LPS animals (48±13% and 161±24%, respectively) above control levels. Immediately after the scan, animals were sacrificed and various organs harvested. In the heart, gamma counting revealed significant increases in radioactivity above both 10 and 30ng/kg LPS above controls (9630±38% and 233±51%, respectively, p<0.05). In the lungs and spleen, only the 30ng/kg LPS mice were significantly above control levels (193±51% and 272±70%, respectively, p<0.05). To determine the specificity of the PBR response, 30ng/kg LPS mice were treated with excess non-radioactive (R)-PK11195. 30 minutes prior to tracer injection. Static planar images revealed that activity in the heart of experimentally inflamed animals was lower than in 30ng/kg LPS animals (decreased 51.5%, p<0.01). Gamma counting revealed that specific tracer activity in the blocked animals was decreased in the lungs (39±7% p<0.023) and spleen (54±80%, p<0.012). These data show that LPS induces specific increases in PBR ligand binding in the lungs and spleen. Further, this study indicates that PBR expression is a promising marker for imaging inflammatory responses. [Supported by grant # ES07062 to TRG]
1742  EFFECTS OF INHALED CARBON BLACK PARTICLE OVERLOAD ON CYTOKINE, OXIDANT, AND MUTATIONAL RESPONSE IN THE LUNG: A SPECIES COMPARISON.
1Central Product Safety, Procter & Gamble, Cincinnati, OH and 2Environmental Medicine, University of Rochester, Rochester, NY.

Increased levels of adenomas and carcinomas in the rat lung have been associated with chronic inhalation to poorly soluble low toxicity particles at levels that cause persistent, marked inflammation. This response appears to be unique to the rat species and has not been observed in mice, or hamsters. Substantial evidence indicates this species specific lung tumor response is a secondary, non-genotoxic, inflammatory mechanism. The resistance of neoplastics during the inflammatory response and their subsequent release of oxidants from these cells have been demonstrated to play an important role in the pathogenesis of lung tumors. To investigate mechanisms underlying species specific lung inflammation and its persistence, female Fisher 344 rats and B6C3F1 mice were exposed to air, 1, 7 and 50mg/m^3 of carbon black for 13 weeks. Serial sacrifices were performed immediately, 3 months and 11 months post exposure. Bronchoalveolar lavage (BAL) was performed and characterized for cell number, type, oxidants and antioxidant levels.

ex vivo mutational analysis of inflammatory cells was evaluated by co-inubating with a lung epithelial cell line (RLE). Additionally, lung tissue was evaluated for gene expression and enzyme activity of various pro- and anti-inflammatory mediators.

There was a dose and time related effect with all the parameters evaluated for both species. Rats showed a greater propensity for generating a pro-inflammatory response while mice demonstrated an increased anti-inflammatory response. These differences in the pro- and anti-inflammatory response may contribute to the apparent species differences in tumor response. Sponsored by the International Carbon Black Association.

1743  COMPARATIVE DOSE-RELATED EFFECTS IN THE LUNGS OF RATS AND MICE FOLLOWING CARBON BLACK INHALATION.
1Environmental Medicine, University of Rochester, Rochester, NY; 2Pediatrics, University of Rochester, Rochester, NY; 3Michigan State University, East Lansing, MI; and 4Proctor and Gamble Company, Cincinnati, OH.

High concentrations of inhaled carbon black (CB) induce overload-associated lung tumors in rats, but not mice or hamsters. The specificity of this response is not related to direct genotoxicity of CB particles, but may be related to species-dependent activation and progression of inflammatory and proliferative processes. In order to examine particle dose-related inflammation, responses to inhaled CB (1, 7, and 50 mg/m^3, 13 wks) in the post-exposure phase have been compared in female F-344 rats and B6C3F1 mice. At the end of exposure, mice had retained more CB per gram of lung in terms of particle surface area than rats; however, clearance was slower in rats such that the CB burden in the high-dose group was higher than the mice 11 months post-exposure. Throughout the post-exposure phase, CB-induced increases in lung weights were comparable in rats and mice. For the low and medium dose groups, increases in total lavageable cells were also comparable in the two species; however, the total cell increase after high-dose CB in rats was greater and more prolonged. Lavage neutrophils increased in both species in response to mid- and high-dose CB; however, the response to the highest dose was -3-10 times greater in rats than in mice. Increases in biochemical indices of inflammation were comparable in rats and mice at the lower doses, but higher in rats at the highest dose. The results suggest that the lowest exposure dose of 1 mg/m^3 CB did not have any adverse effects in either species. In addition, rats appear to have greater and more prolonged inflammation in response to high-dose CB as well as retain CB longer. Funded by the International Carbon Black Association.

1744  INVESTIGATIONS ON THE INFLAMMATORY AND GENOTOXIC LUNG EFFECTS OF TWO TYPES OF ULTRAFINE TITANIUM DIOXIDE - UNTREATED AND SURFACE TREATED.
B. Rehn, P. Sellek, J. Bruch and M. Maier.
1Institute of Hygiene and Occupational Medicine, University of Essen Medical School, Essen, Germany and 2Aerofil & Silanes, Degussa AG, Hanau-Wolfgang, Germany. Sponsor: J. Bartter.

TiO_2 is considered to be toxicologically inert, at least under non-overload conditions. There is discussion about enhanced toxicity of ultrafine particles, independent of the particle chemistry. Therefore, we investigated inflammatory and genotoxic lung effects of two types of ultrafine TiO_2 at low doses relevant for working environments. Female Wistar rats were exposed by instillation to a single dose of 0.15, 0.3, 0.6, and 1.2 mg of ultrafine TiO_2 (P25, untreated) or ultrafine TiO_2 (1805, surface treated) particles suspended in 0.2 ml of physiological saline containing 0.25% [wt] Tween 80. Animals were exposed by instillation with the vehicle only or with a single dose of 0.6 mg quercetin (0.25 ml, 3, 24, and 90 days after exposure), 10 rats per group were sacrificed. In 5 animals bronchoalveolar lavage was performed and inflammatory parameters (cells, protein, TNF-alpha, fibronectin, surfactant phospholipids) were measured. The other animals’ lungs were inflated with freezing medium, removed and stored in liquid nitrogen. For immunohistochemical staining, 8 μm frozen sections from the left lobe were cut and stored at -80°C. The sections were used for detection of 8-oxoguanine by a polyclonal antibody. Using a secondary antibody fluorescence labelled antibody, 8-oxoguanine was quantified by image analysis. In the quiescent exposed animals, a strong progression in lung inflammatory response was observed. 90 days after exposure, a slight but not significant increase in the amount of 8-oxoguanine in the DNA of lung cells was detected. In contrast, animals exposed to both types of TiO_2 showed no signs of inflammation 90 days after exposure. The level of 8-oxoguanine as a marker of DNA damage was comparable to vehicle control, even in the highest dose. The results indicate the inert type of both titanium oxides because neither any inflammatory nor genotoxic effects could be observed in the hygienic relevant range of doses.

1745  INFLAMMATORY TRANSCRIPT AND PROTEIN PRODUCTION BY CULTURED HUMAN KERATINOCYTES FOLLOWING SULFUR MUSTARD (SM) EXPOSURE.
Applied Pharmacology, USAMRICD, Aberdeen Proving Ground, MD.

Human skin exposure to SM occurs initially without pain; however, over time, SM produces a painful burn with the loss of adherence of the basal keratinocyte layer to the dermis, which progresses to form large coalescing fluid-filled blisters. The primary changes occurring within the epidermal basal keratinocyte layer producing this loss of attachment are not known. However, in human skin, a strong delayed inflammatory response occurs, which we believe enhances blister formation and tissue damage. We were interested in defining the specific inflammation transcript and protein changes produced by cultured human epidermal keratinocytes (HEK) following SM exposure. To focus these studies, we initially probed cDNA blots and produced subtraction libraries with cDNA generated from polyA transcripts isolated at various times (0.5, 1, 2, 4, 8, and 16 hr) after HEK exposure to SM. HEK exposure for these studies was performed either at 25 μM SM, an estimated sub-vesicating skin exposure, or at 200 μM, a vesicating exposure of SM. Using both techniques, we identified interleukin-8 (IL-8) as the predominant pro-inflammatory transcript up regulated in response to 200 μM SM. Transcripts also found using cDNA arrays were macrophage inflammatory protein-2α (MIP-2α) and tumor necrosis factor-α (TNF-α). None of these transcripts was increased at the 25 μM SM exposure. Real-time PCR studies using an exposure range of 12.5-200 μM SM have identified transcript and protein increases in IL-8, TNF-α and MIP-2α as early as 1-2 hrs for dose 100 and 200 μM, and the transcripts increase dramatically over time. Studies by E. J. Kanerva and J. L. Schlegel showed a 30% decrease in protein production for 200 μM SM exposure. These inflammatory transcript and protein data correlate well to doses estimated to blister skin and define the specific proteins that may be participating in the inflammatory events produced by HEK in exposed skin.

1746  FOUR WEEK INHALATION TOXICITY STUDY WITH NYLON RFP IN RATS.
D. B. Warheit, K. L. Reed, T. Webb and G. L. Kennedy. DuPont Hadley Laboratory for Health and Environmental Sciences, Newark, DE.

Groups of male rats were exposed, nose-only to aerosols of Nylon RFP (respirable-sized fiber-shaped particulates) for 4 weeks at concentrations of 0, 4, 15 and 57 f/cc (ratio of RFP: particles 1:10-20). The mean gravimetric counts were 0.6 low, 2.7 (mid) and 19.6 (high) level mg/m^3. The samples containing Nylon RFPs were prepared using flocks of rats submerged in a vessel containing the RFPs. Subsequently, the lungs of sham and Nylon-exposed rats were examined at 24 hrs, 1 week, and 1, 3, 6 and 12 months postexposure (pe) using bronchoalveolar lavage (BAL) and/or lung tissue analyses. Results showed that retained mean lung burdens at 24 hrs pe were 1.8±0.07 RFP (high level), 3.4±0.06 RFP (mid level) and 4.8±0.05 RFP (low level). Mean lengths and diameters of the Nylon aerosol were 8.9±1.6 μm, respectively. Six months pe, the retained lung burdens were 8.9±0.05 RFP(high), 1.9±0.05 RFP (mid), and 1.7±0.05 RFP (low level). There were no significant increases in lung weights or indications of pulmonary inflammation/cell proliferation in Nylon-exposed animals compared to controls - based on BAL cell differentials, BAL fluid analyses, BrdU cell proliferation (airway and bronchial) and chemotaxis activity. Greater than 90% of the alveolar macrophages recovered by BAL (high level) contained Nylon RFP or
nonfibrous particulates within their cytoplasm. Interim histopathological analyses have thusfar (thus 6 months pe) revealed no adverse lower pulmonary or upper respiratory effects. These results indicate that 4-week inhalation exposures in rats to concentrations as high as 57 Fl/cc Nylon RFP produced no significant adverse pulmonary effects. (Study funded by the American Fiber Manufacturers Association).

1747 REMOVAL OF SOLUBLE METALS FROM RESIDUAL OIL FLY ASH BY CHELEX PROTECTED RATS FROM INCREASES IN MORTALITY AND LUNG INJURY AFTER INFECTION.

J. R. Roberts, M. D. Taylor, A. B. Lewis and J. M. Antonini

Inhalation of residual oil fly ash (ROFA) impairs lung defense in susceptible populations. Bioavailability of soluble transition metals appears to play a key role in compromised lung defense caused by ROFA exposure. We evaluated the effect of soluble metals on lung defense in animals pre-exposed to ROFA followed by pulmonary challenge with a bacterial pathogen. ROFA was collected from Boston Edison Co., Everett, MA, suspended in saline, incubated for 24 hr at 37°C, and separated into soluble (ROFA-Sol) and insoluble (ROFA-Insol) fractions. A portion of ROFA-Sol was treated with the metal-binding resin, Chelex, for 24 hr at 37°C. None of the fly ash wass intratracheally instilled at day 0.1 mg/10 g body wt of ROFA-Insol, ROFA-Sol, saline, Chelex or ROFA-Sol + Chelex. At day 3, 3×10^5 Listeria monocytogenes were intratracheally instilled into rats from each treatment group. At days 6, 8 and 10, left lungs were removed, homogenized, cultured, and colony forming units (CFUs) were counted to assess bacterial clearance. Treatment with ROFA-Sol before infection led to a marked increase in lung injury at all time points after inoculation, and a 50% increase in mortality when compared to control. On day 6, a 34-fold increase in the number of bacterial CFUs was observed for the ROFA-Insol group when compared with saline control. By day 10, the ROFA-Sol group had 3063 times more lung CFUs than saline control, indicating its inability to effectively respond to the infection. None of the other groups showed significant differences in bacterial clearance. In summary, exposure to ROFA-Sol suppressed the lung response to infection. Upon removal of soluble metals from ROFA, there was no alteration in lung defense mechanism. Soluble metals present in ROFA may play a key role in increased susceptibility to pulmonary infection in exposed populations.

1748 GENERATION OF METAL-INDUCED REACTIVE OXYGEN SPECIES BY RESIDUAL OIL FLY ASH.


Inhalation of residual oil fly ash (ROFA) increases pulmonary morbidity in exposed weanlings. We examined the role of reactive oxygen species (ROS) in ROFA-induced lung injury. ROFA was collected from Boston Edison Co., Everett, MA. ROFA (ROFA-total) was suspended in saline, incubated for 24 hr at 37°C, centrifuged, and separated into soluble (ROFA-sol) and insoluble (ROFA-insol) fractions. The generation of ROS from the ROFA samples was measured by electron spin resonance (ESR). Sprague-Dawley rats were intratracheally instilled with saline, ROFA-total, ROFA-sol, or ROFA-Insol (1 mg/100 g body weight). Lung tissue and bronchial lavage cells were harvested at 4, 24, and 72 hr after instillation. Chemiluminescence (CL) of recovered cells was measured as an index of ROS production, and tissue lipid peroxidation was assessed to assay oxidative injury. Using a spin trap (DMPO), hydroxyl radicals were detected in the ROFA-total sample. Significantly more hydroxyl radicals were measured in ROFA-sol as compared to ROFA-insol. Superoxide anion and hydrogen peroxide were implicated in the production of the hydroxyl radicals. After treatment with the metal chelator, deferoxamine, the production of hydroxyl radical was greatly diminished. None of the ROFA samples had an effect on CL or lipid peroxidation at 4 hr. Treatment with ROFA-total and ROFA-sol caused significant CL increases at day 0.05 (1.0 μmol CL (at 24 hr) and lipid peroxidation (at 24 and 72 hr) when compared to control. ROFA-sol significantly reduced (p<0.05) CL production at 72 hr after treatment and had no effect on lipid peroxidation at any time point. In summary, ROFA, particularly its soluble fraction, generated a metal-dependent hydroxyl radical as measured by cell-free ESR. However, cellular oxidation production and tissue injury were observed mostly with the ROFA-total and ROFA-sol particulate forms. ROS generated by ROFA-sol as measured by ESR appear not to play a major role in the lung injury caused after ROFA exposure.

1749 REMODELING IN HUMAN LUNG IN RELATION TO PARTICLE AND METAL CONTENT.


Little data exists about particle/metal burden in human lung and associated health effects. Remodeling of the terminal and respiratory bronchioles occurs at sites of particle deposition. We extend these findings providing data on particle and metal content in relation to indicators of effect classified by standard diagnostic criteria for 1) chronic bronchitis 2) asthma 3) mineral dust disease and 4) smoking-related disease. Lung autopsies (non-respiratory related deaths) were examined from 40 males from the Central Valley of California. Scanning electron microscopy was used to determine particle type (No, particles/mg tissue) and inductively coupled plasma mass spectrometry for metal analysis. Lung samples with and without indicators of effect were compared. Significant (p<0.05) fold increases in no. of particles were observed for indicators of mineral dust disease: (40x) lymph node fibrosis; (2.0x) smoking related disease (2.2x). No. of particles were (1.6x) lower in asthmatic lung samples. Recently published analyses on blood markers were not presented. Similar findings can contribute to predicting health effects and elucidating the mechanisms of particle induced lung injury. Our data support the association between particle exposure and increased risk of lung disease. Microdissection, histology, evaluation of tissue changes and measurements of internal particle/metal burden provide a means toward establishing dose levels and anatomical sites in human lung required to produce adverse health outcomes.

1750 CARBONYL CONTENT OF DIESEL EXHAUST FROM TWO SOURCES AND POSSIBLE IMPLICATIONS FOR CELL RESPONSES.

J. G. Stohwuermer, L. A. Dailey and M. C. Madden

Human Studies Division, USEPA, Chapel Hill, NC.

Diesel exhaust is known to cause health effects including lung inflammation and altered immunological parameters. The diesel exhausts used in our studies were collected from two sources of diesel exhaust: a bus and a heavy-duty diesel engine. Previous work has shown that DE5A causes more cytotoxicity and greater increases in levels of inflammatory mediators in cultured human airway epithelial cells than DE2A. Some carbonyls are cytotoxic to epithelial cells and increase inflammatory mediator production. We therefore examined whether the carbonyl content of the exhausts were involved in the epithelial cell responses to diesel exhaust exposure. A method was developed for determination of concentration of C2 to C2 aldehydes by HPLC/MS. Diesel exhausts were derivatized with 2, 4-dinitrophenylhydrazone and derivatives isolated by hexane extraction and evaporation under nitrogen. Using quantitation of the peak at 365 nm in the UV/VIS spectra as an index of the total concentration of carbonyl compounds in the reconstructed extracts. DE2A was found to contain a >10x higher level of total carbonyl compounds than DE5A, indicating an inverse correlation between toxicity and total carbonyl content. Reconstructed extracts were injected into an HPLC/MS with a C18 column using ESF negative ion detection with selective ion monitoring. HPLC/MS results were consistent with the UV/VIS result in that levels of polar aldehydes were lower in DE5A than DE2A. Several peaks eluted at 5 min in DE2A but not in DE5A. These data suggest that diesel-derived carbonyls of intermediate polarity may be important in epithelial cell-mediated responses. These observations underscore the importance of sensitive and selective analytical methods such as HPLC/MS in linking components of complex mixtures to biological effects. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

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1751 DIFFERENTIAL PERSISTENCE OF ALVEOLOSES AND EPITHELIAL Proliferation in the Lung of Rats and Mice Exposed to Carbon Black.

M. Ambo, J. R. Hartlma, J. K. Golding, A. C. Elder, F. K. Driscoll and G. Oberdorster

Michigan State University, East Lansing, MI. University of Rochester, Rochester, NY and Proctor & Gamble Pharmaceuticals, Mason, OH.

Rats, but not mice, chronically exposed to high levels of airborne carbon black particles (CB) or other low toxicity particles develop lung tumors in association with chronic alveolar inflammation and epithelial proliferation. The purpose of the present study was to determine the postexposure persistence of alveolitis and type II cell
proliferation in the lungs of rats and mice similarly exposed to Cb. Female F344 rats and B6C3F1 mice were exposed by inhalation to three concentrations of Cb (1, 7, 50 mg/m³) for 6 hr/day, 5 days/wk, for 13 wk. Rodents in each group were sacrificed one day or 13 wk postexposure (PE). The left lung lobe of each animal was processed for light microscopy. Nonglomerular cell densities of alveolar type II cells and neutrophils were determined using image analysis and standard morphometric techniques. Compared to air-exposed controls (0 mg/m³ Cb), rats exposed to Cb had a dose-dependent increase in nonglomerular cell densities of neutrophils (alveolitis) and alveolar type II cells (epithelial hyperplasia). Cb-induced type II cell proliferation persisted throughout 13 wk PE only in the lungs of rats that were exposed to 50 mg/m³ Cb. In contrast, no or minimal Cb-induced alveolitis was evident in Cb-exposed mice at one day or 13 wk postexposure. A minimal increase in the number of alveolar type II cells was evident only in mice exposed to 50 mg/m³ Cb and sacrificed 13 wk PE. These results indicate that rats, but not mice, chronically exposed to high airborne concentrations of Cb develop severe and persistent alveolitis and epithelial proliferation for at least 13 wk PE. No persistent lung lesions were observed in mice or rats exposed to 1 mg/m³ Cb. (Research sponsored by the International Carbon Black Association)

1752 PARTICLE NUMBER AND SURFACE CHARGE PREDICT THE BIOLOGICAL ACTIVATION OF HUMANS. BRONCHIAL EPITHELIAL CELLS EXPOSED TO PARTICULATE MATTER.

B. Veronesi², G. Wei¹, W. Barnett and M. Oortgiesen.¹. NIEHS, USEPA, Research Triangle Park, NC;¹CLC, Novocea, CA and ¹Carlo Research Triangle Park, NC.

Exposure to particulate matter (PM) produces a uniform degree of mortality in exposed populations, in spite of its diverse sources. This suggests a common mechanism of action to explain its initial toxicity. The present study relates certain physicochemical characteristics (i.e., size, number, surface charge, acidity) of PM particles to their biological activation (i.e., increases in intracellular calcium, [Ca²⁺]), IL-6 release) in human, immortalized, tracheal bronchial epithelial cells (BEAS-2B). Ambient urban, volcanic, occupational and industrial PM were examined in suspension and after being passed through a 0.22 μm pore size, cellulose-acetate filters. The size, number and surface charge (i.e., zeta potential) of PM suspensions and their microfiltrates were measured using ZetaPALS technology. Linear regression analysis correlated the IL-6 release caused by field PM and their microfiltrates with their physicochemical characteristics. All of these, the surface charge (i.e., zeta potential) of particles in the suspended and microfiltrate fractions strongly correlated with the IL-6 released from their respective suspensions. Similarly, the counts of microfiltrate particles also significantly correlated with IL-6 release associated with these fractions. To test whether the surface charge alone could stimulate cytokine release, polyurethane beads (SPM) of low and moderate surface potentials were obtained. Exposing cells to 12-60 μg/cm² concentration of SPM produced IL-6 release similar to PM, which was sensitive to CPZ exposure, an antagonist of the VR1 receptor. RT-PCR indicated that the charged SPM activated the acid sensitive, VR1 receptor of the BEAS-2B cells. Together, these data suggest that the surface charge of particles can stimulate a measurable biologic response in human respiratory tract cells. (This abstract has been reviewed by NIEHS but does not necessarily reflect EPA policy.)

1753 A TYPICAL PATH MODEL OF TRACHEOBRONCHIAL CLEARANCE OF INHALED PARTICLES IN RATS.

E. C. Kimmel, J. E. Rebrout and R. J. Carpenter. Naval Health Research Center Toxicology Detachment, WPAFB, OH.

A typical path model of tracheobronchial (TB) particle clearance was developed using previously reported deposition model airway anatomy (Kimmel et al., 1998, Carpenter and Kimmel, 1999). Particle transport velocities for a typical airway in each generation were extrapolated from mean tracheal mucous transport velocities (TMV) using allometric equations describing the relationships between TMV and airway surface area, diameter, and length. Published interspecies allometric equations were modified to use theoretical (dimensional) exponents, indicating a greater degree of sameness, for interspecies extrapolations. Multi-component clearance rate equations describing a typical clearance path from each generation of airways were developed. Consideration was given to conservation of mucous flow between airways. Overall TB region clearance was calculated by applying these clearance rate equations to the predicted fractional of aerosol particles deposited in corresponding airways generations. Predicted short-term clearance half-lives were 4.45, 6.42, and 20.97 hrs for aerosols in which theoretical deposition was 100, 80, and 50 % distributed equally throughout the TB region. Although these initial predictions were in general agreement (within 15%) with published data they systematically overpredicted clearance. Adjustments of model clearance velocities for airway size dependent differences in mucous thickness and density and comprehensive comparison of model predictions with published data will be presented.

1754 THE EFFECT OF SIZE FRACTIONATED PARTICULATE MATTER ON HUMAN AIRWAY EPITHELIAL CELLS IN VITRO.

L. A. Dailey¹, C. Sioukas¹, J. M. Seulku¹, S. Becker and B. B. DeLwin¹.¹NIEHS, USEPA, Research Triangle Park, NC and ¹Civil and Environmental Engineering, USC, Los Angeles, CA.

Epidemiologic studies have demonstrated a compelling causal relationship between episodes of high concentrations of particulate matter (PM) and adverse health effects to the human respiratory system and cardiovascular system, as evidenced by increased hospitalizations and mortality. Many factors have been implicated in the toxicity of PM, including particle size, number and constituents. Advances in technology now allow researchers to simultaneously collect, concentrate, and size fractionate coarse, fine, and ultrafine ambient air particles. We have collected Chapel Hill ambient PM in the following size ranges: coarse (CS) 2.5-10 micron, fine (FN) 0.1-2.5 micron, and ultrafine (UF) <0.1 micron and examined their effects on human primary airway epithelial cells in vitro. Cells were exposed to 25, 100, and 250 μg/ml doses of each PM fraction for 2 and 24 hours. LDH and the inflammatory cytokine IL-8 were measured. Preliminary studies show that all three fractions elicited a dose-dependent response at 24 hours. When airway epithelial cells were exposed to different PM fractions on an equal mass basis, CS and UF fractions appeared to induce a larger cytokine response than FN particles. At 250 μg/ml, the highest dose examined, cells exposed to CS PM had approximately 4.3 fold greater IL-8 production than untreated cells; cells exposed to UF had 3.7 fold increased IL-8, while cells exposed to FN PM had 2.5 fold increased IL-8 compared to untreated cells. Similarly, human alveolar macrophages exposed 18 hours to 10 μg/ml CS and FN PM showed a far greater IL-6 cytokine production in response to coarse PM than fine PM (81.2 vs 7.8 fold increases compared to controls). These studies suggest that coarse PM particles can induce increased inflammatory mediators in important lung cell types. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1755 cDNA MICROARRAY ANALYSIS OF GENE EXPRESSION IN RAT ALVEOLAR MACROPHAGES IN RESPONSE TO ORGANIC EXTRACT OF DIESEL EXHAUST PARTICLES.

E. Koike¹, S. Hirano¹ and T. Kobayashi¹.¹Department of Medicine, University of Tsukuba, Tsukuba, Japan;²Research Center for Environmental Risk, National Institute for Environmental Studies, Tsukuba, Japan and ²Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Japan.

Diesel exhaust particles (DEP) induce pulmonary diseases including asthma and chronic bronchitis. The comprehensive evaluation has been required to know the effects of air pollutants including Department on the lung diseases. Alveolar macrophages (AM) encounter and phagocytose Department in the alveolar space and their biological responses are implicated in Department-induced pulmonary diseases. Therefore, we investigated the gene expression in rat AM after exposure to Department extract using a cDNA microarray. AM were prepared from male Sprague-Dawley rats (8-10 w) by bronchoscoelar lavage. The Department extract was prepared using dichloromethane. AM were pre-cultured for 20 hr and exposed to 10 g/ml Department extract for 6 hr. Total RNA was extracted from AM using TRIZOL. cDNA probe synthesis and cDNA microanalysis were carried out using an Atlas Pure Total RNA Labeling System and Atlas Rat Toxicology Array II (CLONTECH) that includes 450 rat cDNAs. The results were further confirmed by northern blot analysis. Transcriptions of 6 genes on the cDNA microarray were significantly elevated by exposure to Department extract. These were heme oxygenase (HO-1 and 2), thioredoxin peroxidase 2 (TDPX-2), glutathione S-transferase θ subunit (GST-θ), NAD(P)H dehydrogenase, and proliferating cell nuclear antigen (PCNA). The antioxidative enzymes such as HO, TDPX-2, GST-θ, and NAD(P)H dehydrogenase may play a role in the pulmonary defense against oxidative stress caused by Department. PCNA may have contributed to the repair of the DNA damage and cell proliferation. Our results suggest that cDNA microarray analysis provides useful information to investigate the biological response to pulmonary toxicants.

1756 EFFECT OF PARTICULATE MATTER UNDER 2,5 mm (PM2.5) AND DIESEL EXHAUST PARTICULATE (DEP) ON RESPIRATORY AND CARDIAC FUNCTION IN SPONTANEOUSLY HYPTERTENSION RATS.

M. K. Uijima¹, B. Murayama and T. Kobayashi¹.¹Department of Medicine, Tsukuba University, Tsukuba, Japan, ²School of Nursing, Miyagi University, Miyagi, Japan and ²Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Japan. Sponsor: S. Hirano.

Epidemiological studies have shown the association between the morbidity and mortality of populations with preexisting cardiovascular disease and concentration of PM2.5. The present study was designed to clarify the effect of installation of
PM2.5 on respiratory and cardiac function in rats with hereditary systemic hypertension. Male spontaneously hypertensive (SH) rats and Wistar Kyoto (WKY) rats, control strain, (11-12 weeks old, 8/group) were used. Rats were operated to insert electrode subcutaneously. One week after, they were anesthetized with halothane, and were installed intratracheally with PM2.5 extract or Department extract (3mg/kg) in DMSO-PE solution. Pulmonary functions and electrical measurement were measured at 3h, 3h, and 24h after installation. R-R interval, power spectra of low frequency (LF) and high frequency (HF) were analyzed from electrocardiogram. LF/HF ratio and HF contents are indices of sympathetic and parasympathetic nervous activities, respectively. Long resistance increased in SH and WKY rats administrated with PM2.5 or Department extract. In SH rats administrated with PM2.5, R-R interval extended, HF increased, but LF/HF did not change. In WKY rats administrated with PM2.5, HF tended to increase, but R-R interval and LF/HF did not change. Department, however, R-R interval, HF, and LF/HF were not changed by Department in both SH and WKY rats. In conclusion, PM2.5 decreased heart rate and increased parasympathetic nervous activity in SH rats. Our results suggest that populations possessing hypertension might be susceptible to PM2.5.

1757

The assessment of carcinogenicity of Man Made Fibers (MMF) in rats by the intrapulmonary (i.p.) route has been proposed as alternative to inhalation studies, which may offer greater sensitivity. We have evaluated the carcinogenic potential of five viscous MMF using this approach; MMF were administered as 2 to 20 weekly i.p. injections to groups of 51 female Wistar rats in a 123 week study. Crocidolite was used as a reference carcinogenic fiber. The tested fibers (designated M, O, P, V, and B detectable in the test) were given to 5 to 10 fibers, corresponding to an injected mass up to 400 - 700 mg. Crocidolite was given at dose levels up to 1 x 10^6 fibers (up to 5 mg). The tested fibers did not produce notable adverse effects on the basis of mortality, clinical signs and body weight, except at the highest dose level. All the fibers produced non-specific fibrous adhesions between abdominal organs, which was unrelated severity; this finding was sometimes associated with chronic peritonitis or hepatocellular necrosis. Interestingly, the incidence of mesotheliomas permitted discrimination between the various fibers: fibers M and O did not induce any mesothelioma at dose-levels up to 5 x 10^6 fibers; fibers P and V induced mesotheliomas in 16% and 28% of animals respectively at 5 x 10^6 fibers; fiber B for which previously published data was also available, induced mesotheliomas in 18% of animals - 90% of the crocidolite-treated animals displayed mesotheliomas at 1 x 10^6 fibers. No fiber-related tumor other than mesothelioma was observed. In conclusion, long-term bioassay in rats with i.p. injection was found to be a suitable and sensitive model which permitted discrimination of the carcinogenic potential of MMF with similar solubility or bioavailability.

1758

Epidemiological studies have linked increased levels of ambient particulate matter (PM) with increased morbidity and mortality, noting higher correlations in persons with cardiopulmonary disease. To investigate this phenomenon, we examined the effects of ROFA (model emission PM) in SH rats (a susceptible disease model). Rats were implanted with radioelements capable of monitoring electrocardiogram (ECG), heart rate (HR), and core temperature (Tc). Animals were divided into four groups and exposed via intratracheal instillation (IT). Observations were performed for 96h post-IT and ventilatory function was examined (Buxco Electronics) for 6h/day on postexposure days 1-4. At 24h and 96h post-IT, subsets of rats underwent bronchoalveolar lavage (BAL) and the BAL fluid was examined for biochemical indices of pulmonary damage and inflammation. The IT exposures were performed to vehicle and low dose ROFA caused no changes in Tc and while mild dose ROFA induced substantial decreases (1.2-2.6°C). ROFA-induced decreases in HR (30-120 bpm) did not return to control levels until 24h post-IT (low dose) or 72h post-IT (mid and high dose). ECG abnormalities (rhythm disturbances, premature ventricular contractions) were observed primarily in the high dose group. Adverse changes in BAL indices mirrored the dose-related changes in physiological parameters, yielding increases in protein, and LDH (24h post-IT >96h post-IT). These studies demonstrate substantial deficits in cardiopulmonary function in SH rats after IT exposure to ROFA PM, along with significant increases in BAP indices of pulmonary injury. Toxicity appears to be greater than that sustained previously in in vitro tests and comparable to that observed in other compromised rodent models. (Abstract does not represent USEPA policy).

1760

Spontaneously hypertensive (SH) rats, a strain with the genetic tendency to develop pulmonary and cardiac disease, are being used as a model to study the health effects of particulate matter (PM). In the present studies male SH rats were subjected to multiple exposures (12 exposures, over a 6 week period, 4/week/day of 1 either filtered air (control), or concentrated air particles (CAPS) from the Research Triangle Park (RTP), NC. Then, primary rat tracheal epithelial (RTE) cell cultures were established from both control and CAPS-exposed rats using an air-liquid interface system. After reaching confluency and maturity, cells were exposed to saline control, or to RTP-PM (PM 1.7-3.7, collected in bulk using a high volume sampler) at either 3.5 or 35 ug/cm² of culture area. Toxicity and cell injury were evaluated by lactate dehydrogenase (LDH) released and albinum translocation (index of increased solute permeability). Cell metabolism changes were evaluated by measuring intracellular glutathione (GSH) and ATP levels. RTE cell exposure to RTP-PM did not induce significant changes in LDH released by RTE cells. However, exposure of RTE cells to RTP-PM at 35 ug/cm² induced a mild increase in apical albumin levels that was greater in cells originating from CAPS-exposed rats than in cells from control-exposed rats. RTP-PM exposures also induced decreases in GSH levels in cells originated from either air- or CAPS-exposed SH rats. RTP-PM exposures induced increases in ATP levels in RTE cells from either air or CAPS-exposed SH rats. Alterations of cellular metabolism by CAPS could contribute to the enhanced responses of cardiopulmonary compromised animals to the effects of ambient air pollutants, however more studies are needed to elucidate the extent of these differences. The contents of this abstract do not necessarily reflect the views and policies of the USEPA.

1761
HEALTH EFFECTS OF CONCENTRATED CALIFORNIA PARTICULATE MATTER IN RATS. K. R. Swint, S. Kim, J. J. Recendes, C. Sioutas and K. E. Pinkerton. ITHEM, University of California, Davis, CA and Civil Engineering, University of Southern California, Los Angeles, CA.

Epidemiological studies have shown that airborne particulate matter (PM) with an aerodynamic diameter less than 10 microns is associated with increased pulmonary disease. However, particles less than 2.5 microns are thought to elicit the greatest
toxicity. PM is a complex mixture of organic and inorganic compounds, but the properties responsible for its effects on the respiratory system remain unknown. During the fall and winter of 2000-01 adult Sprague-Dawley rats were exposed in Fresno, CA to filtered air or combined ultrafine and fine fractions of PM measured approximately 20-fold over ambient levels in 6 separate experiments. Exposures were for 4 hours per day for 3 consecutive days. PM was concentrated while preserving chemical composition, size distribution and surface morphology. PM mass and particle numbers were measured continuously during exposure. Chemical analysis of the concentrated PM was conducted for each study. The mean mass concentration of particles ranged from 200 to 500 micrograms/cubic meter. PM was enriched primarily with nitrate, organic and elemental carbon, and metals. Bronchoalveolar lavage (BAL) was performed on each group of rats following exposure. Viability, total cells, and numbers of macrophages, neutrophils and lymphocytes in the BAL was determined. Viability of BAL cells from rats exposed to concentrated PM was significantly decreased during 4 of 6 weeks, compared to rats exposed to filtered air (P<0.05). Total BAL cells were increased during 1 week of the 6-week study, while macrophages increased during 1 week and neutrophils during 2 weeks. The decreased cell viability in PM-exposed rats is indicative of cell membrane damage, possibly through oxidative stress catalyzed by transition metals. The increased neutrophils in PM-exposed rats indicates a mild inflammatory response. These observations suggest that exposure to enhanced concentrations of ambient particles in Fresno, CA is associated with significant effects in the lungs of healthy adult rats.

1762 EVALUATION OF TOXICITY BY OIL FLY-ASHES IN RAT ALVEOLAR EPITHELIAL CELLS: ROLE OF FENTON DRIVEN HYDROXYL RADICAL GENERATION AND PARTICLE INTERACTION.


Leachable transition metals are considered to be important mediators in the biological effects of ambient particulate matter (PM) and model particles such as oil fly ashes (OFA). We determined metal driven Fenton activity in 10 OFA in the presence of hydrogen peroxide using electron spin resonance (EPR) and a suitable spin-trap (DMPO) and the ability to induce 8-hydroxydeoxyguanosine (8-OHdG) in nucleic DNA using immunodot blot. In addition, the toxicity of the OFA suspensions and supernatants was determined in a rat alveolar type II cell line using lactate dehydrogenase (LDH) (membrane damage) or conversion of MTT (mitochondrial function). Suspensions of OFA were consistently more toxic than their filtrates obtained by centrifugation and 0.1 μm filtration. Correlations between both cytotoxicity assays were seen using OFA suspensions (r=0.862) but not for the filtrates. The DMPO-OH generation of filtrates in water did correlate well with the cytotoxicity assessed by MTT conversion of the filtrate (r=0.820) and the formation of 8-OHdG (r=0.57).

Together these findings suggest that the different fractions of OFA exert cytotoxicity through different mechanisms. This is supported by different effects of filtrate and suspensions on 8-OHdG formation and MTT resorbtion. The water-soluble Fenton-active fraction seems to affect mitochondrial competence (MTT), while the membranolytic effects seem to be determined by the total suspensions. (This abstract does not reflect USEPA policy).

1763 COMPARISON OF ASBESTOS FIBER CONTENT IN LUNGS OF CANCER PATIENTS WITH NORMAL KOREAN SUBJECTS WITH NO KNOWN OCCUPATIONAL ASBESTOS EXPOSURE HISTORY.

J. Yu, K. Saka, N. Hisanaga, H. Chang, Y. Lee, J. Kwon, H. Jang, and H. Chung, Center for Occupational Toxicology, Korea Occupational Safety & Health Research Institute, Daegu, South Korea, Dept. of Pathology, Kumin University, Daegu, South Korea, Nagoya City Public Health Institute, Nagoya, Japan, National Institute of Industrial Health, Kawasaki, Japan, Dept. of Preventive Medicine, Chung-Ang University, Seoul, South Korea and National Institute of Scientific Investigation, Busan, South Korea.

To evaluate the effect of environmental asbestos exposure on inducing lung cancer, pulmonary fiber contents of both asbestos and non-asbestos types were evaluated in 36 normal Korean subjects and 37 lung cancer patients with no known occupational history of asbestos exposure. Pulmonary fiber contents were analyzed by transmission electron microscopy with energy dispersive X-ray analysis after using the low temperature ashing procedure. Chrysotile fiber was the major fiber type found in the lungs of Korean subjects. Asbestos fiber concentrations found in the lungs of normal 25 males and 11 females were 0.23 x 106 fibers/g of dry lungs and 0.15 x 106 fibers/g of dry lung tissue, respectively. Asbestos concentrations found in the lungs of cancer patients were 0.16 x 106 fibers/g of dry lungs for 31 males and 0.45 x 106 fibers/g of dry lungs for 6 females. There was no statistical difference in pulmonary asbestos content between normal and lung cancer patients.

1764 YOU CAN'T DILUTE FRESH COMBUSTION EMISSIONS WITHOUT ALTERING THEIR COMPOSITION.

J. D. McDonald, Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM, Sponsor: J. Maurer.

Recent research has shown that the dilution level can affect both the particle size distribution and composition of diesel exhaust. This may be important in inhalation toxicology because lung deposition patterns may differ with changes in particle size distribution, and changes in chemistry can lead to non-linear dilutions of all constituents of combustion material such as diesel exhaust. Data reported here were generated during a diesel exhaust inhalation toxicology study conducted at Lovelace Respiratory Research Institute under the auspices of the National Environmental Respiratory Center (NERC). As part of this research, the size distribution and chemical composition of particulate material was investigated at four different dilutions of exhaust. Dilutions with scrubbed ambient air were set to achieve diesel particle concentrations of 30, 100, 300, and 1000 microgram per cubic meter. Particle size distribution, including number count and mass distribution, were characterized at all exposure levels along with the chemical composition of the particulate material. Particle mass distribution centered at approximately 150nm for all exposure levels, but number distribution was different at all levels, with the highest dilution level (lowest mass) producing higher amounts of particles in the "nanoparticle" range (<100nm). Particle composition was found to change most at the more concentrated levels, with chemical interactions between nitrogen oxides and ammonia producing higher relative levels of ammonium nitrate/ammonium sulfate particles at low dilutions levels. Research conducted by the National Environmental Respiratory Center (NERC) with support from multiple government and industry sponsors, including the USEPA. This abstract is not intended to represent the views or policies of any NERC sponsor.

1765 EFFECT OF ULTRAFINE CARBON BLACK EXPOSURE ON RESPIRATORY SYNCYTIAL VIRUS INFECTION IN MICE.

A. L. Lambert, M. P. DeLorme, F. S. Fratti, J. B. Mangum and J. L. Everett, CHI Center for Health Research, Research Triangle Park, NC.

Epidemiological studies have indicated that exposure to elevated levels of ultrafine (UF) particulate matter exacerbates a variety of pulmonary diseases, including asthma, bronchitis, and viral infections. Respiratory syncytial virus (RSV) is the most important etiological agent in respiratory diseases of infancy, the major cause of bronchiolitis and pneumonia in infants under 1 year of age, and may lead to the development of asthma in childhood. RSV also contributes to the development of pneumonia in the elderly and the immunocompromised. To ascertain whether particle exposure modulates the immune response to RSV, we developed a BALB/c mouse model of RSV infection that mimics the pathophysiologic and immunologic features of RSV disease in humans. For the study, 8-week-old female BALB/c mice were infected via the trachea with either 40 μg carbon black (CB) particles or saline vehicle. The following day, mice were instilled with either 10 μl RSV or uninfected media. End points were examined 1, 2, 4, 7, and 10 days after RSV infection. Airways hypersensitivity and total IgA levels in the serum were increased in RSV-infected animals compared to controls and were unaltered by CB exposure. RSV antigen titers in the bronchoalveolar lavage fluid (BALF) and lung homogenates were reduced in RSV-CB-treated mice relative to RSV alone. On days 1 and 2 of infection, TNF-α protein was reduced in the BALF of RSV-CB mice, on day 4, there was a significant reduction in BALF lymphocyte numbers, which correlated with a reduction in INF-γ protein in the BALF of RSV-CB mice compared with RSV alone. Ribonuclease protection assays of lung tissue showed reductions in RANTES, eotaxin, MCP-1, MIP-10, MIP-1β, and IFN-γ levels in IL-6, IL-10, and IL-15 message in RSV-CB mice compared to RSV alone. These data indicate that preexposure to CB particles may suppress alveolar macrophages and the pulmonary immune response to RSV infection, leading to accelerated viral clearance and less severe disease.

1766 ROLE OF ASSOCIATED TRANSITION METALS IN THE IMMUNOTOXICITY OF INHALED PARTICULATE AIR POLLUTION.

I. T. Zellner, K. Schmerhorn, G. Cho, C. Prophues, M. D. Colom and R. B. Schinsinger, Environmental Medicine, New York University School of Medicine, Toronto, NY.

Epidemiological studies demonstrate that pneumonia contributes to increased mortality among individuals exposed to particulate matter (PM). This suggests that PM acts as an immunosuppressant that undermines normal lung immu-
NITRIC OXIDE GENERATION BY CULTURED RAT HEPATOCYTES DURING SIMULATED ISCHEMIA.


BACKGROUND: Nitric oxide (NO) is an important regulator of biological functions that has also been linked to cellular injury. NO can be generated during ischemia/reperfusion, but mechanisms of NO formation in ischemic hepatocytes are not known. The AIM of this study was to investigate the effect of ischemia on the generation of NO in hepatocytes. METHODS: Rat hepatocytes were cultured overnight and loaded with 10 μM DAF-DA dye, an NO specific fluorescent probe, for 30 minutes at 37°C in Krebs-Henseleit HEPES (KHH) buffer at pH 7.4. After washing three times with cold KHH, hepatocytes were further incubated for 15 minutes at 37°C to complete deesterification. The cells in warm KHH were then incubated in air (normoxia) or argon (ischemia) at either pH 6.2 or at pH 7.4 for up to 4 h. Changes in DAF-DA fluorescence were monitored in a fluorimeter and computer as an oxygen consumption rate plate reader. In other experiments, 5 mM arginine, 1 mM of the NO synthesis inhibitor N-nitro-arginine methyl ester hydrochloride (L-NAME), or 100 μM of the NO donor S-nitroso-N-acetylpenicillamine (SNAP) was included in the incubation medium. Confocal images of DAF-DA fluorescence were collected with a Zeiss LSM 410 microscope. RESULTS: NO formation, as revealed by DAF-DA fluorescence, increased during ischemia at pH 6.2 but not at pH 7.4. NO production during 4 h at pH 6.2 was about 10-fold greater than during 4 h of normoxia at pH 6.2. Incubation of hepatocytes with arginine or L-NAME did not increase or decrease NO formation during ischemia, although SNAP rapidly enhanced DAF-DA fluorescence both in normoxic and ischemic cells at either pH. Confocal imaging confirmed a dramatic increase of intracellular DAF-DA fluorescence during ischemia but not during normoxia. CONCLUSION: Anoxia to hepatocytes under acidic conditions enhanced NO formation. The oxygen-independent mechanism by which NO is formed during ischemia appears not to involve nitric oxide synthase. The contribution of NO formation to ischemic hepatic cellular injury remains to be determined.
data indicate that in P and PCN rats, hepatic Mnp2 expression is translatonally regulated.

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**1771**

**INDUCTION OF HEMOXYGENASE 1 (HO-1)**

**PROTECTS AGAINST TNF-α-INDUCED APOPTOSIS AND NEUTROPHIL-MEDIATED NECROSIS IN THE LIVER.**

'Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR and Pathology, University of Texas Health Science Center, Houston, TX.

**HO-1** is a stress-response enzyme, which can protect against oxidative tissue injury. We previously demonstrated the critical role of reactive oxygen in the model of galactosamine/endotoxin-induced apoptosis and neutrophil-mediated liver cell necrosis ([Hepatol. 29: 443-450, 1999]). To investigate potential protective role of HO-1, C3HeB/Fe) mice were pretreated with 5 or 15 mg/kg COsal-tetraproporphin IX (CoTP). This treatment dose-dependently induced HO-1 in non-parenchymal cells and in hepatocytes as assessed by immunohistochemistry and Western blotting of isolated cell fractions. Injection of 700 mg/kg galactosamine and 0.1 mg/kg endotoxin (LPS) caused hepato-cellular apoptosis as indicated by a 10-fold increase of caspase-3 activity and procaspase-2 processing at 6 h. Large numbers of neutrophils were present in sinusoids and portal venular vessels at that time. Following transmission, these neutrophils caused additional cell injury as indicated by high levels of ALT (3720 ± 920 U/L) and necrosis (40-50%) at 7 h.

Both doses of CoTP significantly attenuated the increase in plasma ALT levels (5 mg/kg: 244 ± 120 U/L; 15 mg/kg: 51 ± 14 U/L) and necrosis (10%) but had no effect on hepatic neutrophil sequestration in either vascular bed. Caspase-3 activity was reduced by 40% and 90% with the low and high dose of CoTP, respectively. Additional treatment with the HO-1 inhibitor SnPP restored the sensitivity to LPS-mediated injury. Cell injury after injection of 700 mg/kg galactosamine/7 µg/kg murine TNF-α was also attenuated with HO-1 induction. CONCLUSION: Induction of HO-1 in nonparenchymal cells and in hepatocytes with CoTP dose-dependently protected against TNF-mediated apoptotic cell death and subsequent neutrophil-induced injury to hepatocytes. The mechanism may involve the increased formation of endogenous antioxidants by HO-1.

**1772**

**THE IMPACT OF PERFLUOROOCYL DERIVATIVES ON PEROXISOME PROLIFERATION AND MITOCHONDRIAL BIOGENESIS.**

J. M. Berthiaume and K. B. Wallace. Biochemistry and Mol. Biology, University of Minnesota, Duluth, MN.

Perfluorocarbons (PFCs), perfluoroalkanesulfonate (PFOS), and substituted perfluorocarboxanilamides are commercial compounds widely used as water and stain repellents, fire retardants, and anti-corrosion agents among many other applications. We recently showed that these compounds disrupt mitochondrial bioenergetics in one of three ways: by stimulating proton conductance to uncouple mitochondrial respiration, as inducers of the mitochondrial permeability transition (MPT), or by causing the nonspecific increase in ion permeability across the mitochondrial membrane. The purpose of this investigation was to screen several members of this class of compounds, PFOS, PFOSA, perfluorocarboxanilamides (FOSA), perfluorocarboxanilamides (PFOSA), ethyl perfluorocarboxanilamides (NeF-POSA), and ethyl perfluorocarboxanilamides (NeF-POSA) to reveal a common structural characteristic responsible for induction of the mitochondrial permeability transition. Each compound was added at concentrations previously shown to alter mitochondrial respiration to a reaction medium containing glutamate/malate energized rat liver mitochondria. Mitochondrial swelling was monitored spectrophotometrically. PFOS, FOSA, NeF-POSA, and NeF-POSA did not induce swelling at any concentration. In contrast, the carboxylated compounds PFOSA, FOSA, and NeF-POSA induced calcium-dependent, cyclosporin A inhibited mitochondrial swelling. The fully substituted amide NeF-POSA is the most potent inducer of the MPT, followed by FOSA, then PFOS. The results indicate that the primary determinant of induction of the MPT is the cyclophilic group, and that it is the inducer of the MPT that is responsible for the observed interference with mitochondrial respiration by these carboxyfluorochemicals. (This work is supported by a grant from the 3M Company).

**1774**

**ACETAMINOPHEN TOXICITY AND APOPTOTIC SIGNALING PATHWAYS IN THE LIVER.**

T. R. Knight and H. Jecšćki. Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.

Recently, it has been shown that a moderately toxic dose of acetaminophen (AAP) interrupts the signaling pathway of Fas receptor-mediated apoptosis (Toyokol. Appl Pharmacol 156: 179-186, 1999). The aim of our study was to investigate the mechanism of this effect. Male C3HeB/FeJ mice received a single dose of AAP (300 mg/kg ip) and/or anti-Fas antibody Jo-2 (0.6 mg/kg, iv). Some animals were treated with allopurinol (100 mg/kg, po) 18 and 11 h before AAP injection. RESULTS: After 90 min of Jo-2 treatment, there was processing of BID and procaspase-3 and a significant increase in liver caspase-3 activity, which is consistent with apoptotic cell death. Treatment with AAP 2.5 h before Jo-2 prevented the increase in hepatic caspase-3 activities and the processing of both BID and procaspase-3. When administered alone, AAP did not cause processing of BID or procaspase-3 but caused significant liver injury. AAP metabolism resulted in a substantial depletion of tissue and mitochondrial glutathione (GSH). However, the mitochondrial GSSG levels increased from 3-5% of total glutathione in controls to 15-25% in 1 h after AAP indicating a significant oxidant stress. Pretreatment with allopurinol prevented the mitochondrial oxidant stress and liver injury due to AAP toxicity but had no effect on Jo-2-mediated apoptosis. Consequently, allopurinol restored hepatocyte sensitivity to Fas receptor signaling in AAP-treated animals. Evaluation of DNA fragmentation with the TUNEL assay showed staining of mitochondria (Jo-2) or centrolateral hepatocytes (AAP). Treatment with AAP eliminated staining of midzonal hepatocytes after Jo-2 injection. Allopurinol restored TUNEL staining of midzonal and prevented staining of centrolateral hepatocytes after combined Jo-2 and AAP administration. CONCLUSION: Metabolic activation of AAP causes mitochondrial dysfunction, which is insusceptible for disruption of Fas receptor-mediated intracellular signaling. These data suggest that maintaining mitochondrial integrity is critical for the execution of receptor-mediated apoptotic cell death pathways in hepatocytes.

**1775**

**MECHANISMS OF PROGRESSION AND REGRESSION OF ACUTE LIVER INJURY.**

'Department of Toxicology, College of Pharmacy, The University of Louisiana at Monroe, Monroe, LA and Pathology Associates Inc., NCTR, Jefferson, AR.

Toxicant-initiated liver injury progresses to liver failure unless newly divided cells obscure progressing injury. To test the hypothesis that progression of injury occurs due to destructive action of cytoplasmic and lysosomal degradative enzymes (death proteins) released from dying cells, the role of calpain, a Ca²⁺-activated cytoplasmic
protease was evaluated. A specific capelin inhibition, N-CBZ-VAL-PHE- methyl ester (CBZ, 60 mg/kg, in DMSO), administered ip 1 h after a lethal dose of CCl4 (3 ml/kg, in corn oil, ip) protected 75% of male S-D rats. Liver injury measured by ALT was 6-fold lower in the rats receiving CBZ. Capelin leakage from dying cells was confirmed by immunochemistry in liver sections at 0 to 150 h after CCl4 injection (2 ml/kg, in corn oil, ip). Capelin appeared in the perinuclear intercellular spaces at 3 h after CCl4 and increased up to 48 h. The isometric growth of the injury. In the CCl4 + capelin treated group, capelin leakage and liver injury were strikingly lower. Plasma capelin activity after CCl4 treatment mirrored the intercellular leakage and progression of injury, whereas no elevation was detected in the uninjured liver 24 hours after capelin administration. Breakdown of the cell envelope by the endogenous inhibitor of capelin, a cytoskeletal protein, and specific capelin substrate, analyzed in liver samples by Western blot, increased at 12 and 48 h after CCl4 treatment. Normal rat hepatocytes incubated with capelin and 1.3 mM Free Ca2+ confirmed that capelin can induce cell death. In contrast, dividing cells remained unaffected by the drug. The endogenous inhibitor of capelin was synthesized by a chemoattractive property of endogenous inhibitors. Immunohistochemically, expression of capelin in newly divided cells with the cholestasis drugs: bromocriptine (BCG, 30 umol/kg) and rose bengal (RB, 60 umol/kg) immediately after APAP administration (500mg/kg, i.p.) Other groups were given mice receiving the non-cholestatic drugs (DBS, 150 umol/kg) amarantus (Am, 300umol/kg) prior to APAP. Controls were given vehicle only. Liver toxicity was assessed 24 h later. Hepatic necrosis was evident at 24 h in control mice receiving APAP as determined by serial dehydrogenase activity and histopathology. Pretreatment with the non-cholestatic drugs, BCG and RB decreased the severity of hepatocellular necrosis induced by APAP. However, the administration of the non-cholestatic drugs DBS and AM did not alter APAP-induced liver damage. APAP-induced glutathione depletion was not altered by pretreatment with any of these drugs. Since cholestatic drugs were able to protect from APAP-induced liver damage while non-cholestatic drugs did not, these data indicate that cholestasis is a contributing factor to the changes in susceptibility to APAP hepatotoxicity produced by some of these cholestatic drugs. (Supported by NIH grant ES00929).

1776 **INDUCTION OF RAT LIVER MULTIDRUG RESISTANCE PROTEIN 3 (MRP3) EXPRESSION FOLLOWING TREATMENT WITH CARBON TETRACHLORIDE (CCL4) AND ACETAMINOPHEN (APAP).**

G. E. Hennig, H. E. Whiteley, A. L. Sinn, N. J. Cherington, C. D. Klausen and J. E. Manautou. *Pharmacological Sciences, University of Connecticut, Storrs, CT and Department of Pharmacological Sciences, University of Connecticut, Storrs, CT.*

Chemically-induced compensatory hepatocellular proliferation results in enhanced potential against subsequent toxicant challenge. Induction of liver plasma membrane ATP-dependent transporters may be one of several mechanisms by which proliferating hepatocytes acquire enhanced resistance to cytotoxicity. The multidrug resistance associated proteins are one family of these transport proteins for organic anions. Within this family, Mdrp3 is known to be inducible. Therefore, the relative abundance of Mdrp3 mRNA transcripts was measured using branched DNA signal amplification. Male Wistar rats received either 900 mg APAP/kg, ip, or 26 ml CCl4/kg, ip, and control rats received vehicle only. Liver samples were collected at 6, 24, 48, and 72 hours after treatment and processed for histopathological analysis and isolation of poly A+ RNA. CCL4 produced 10-40% liver necrosis in the 6, 24, and 48 hours samples and 0-10% necrosis in the 72 h samples. APAP produced 10-40% necrosis at 24 h, 0-40% at 48 h, and 0-50% in the 72 h samples. Mdrp3 mRNA expression was significantly increased at 6, 24, and 48 hours following CCl4 administration and at 72 hours after APAP administration. In conclusion, induction of Mdrp3 during compensatory hepatocellular proliferation may be important to the enhanced resistance to chemical-induced cytotoxicity in this model. (Supported by NIH grant ES10093).

1777 **PROTECTION AGAINST ACETAMINOPHEN HEPATOTOXICITY BY CLOFIBRATE PRETREATMENT: EFFECT OF CATALASE INHIBITION.**

C. Chen, G. E. Hennig, H. E. Whiteley and J. E. Manautou. *Pharmacological Sciences, University of Connecticut, Storrs, CT and Department of Pharmacological Sciences, University of Connecticut, Storrs, CT.*

Mice pretreated with the peroxisome proliferator clofibrate (CBF) are highly resistant to acetaminophen (APAP)-induced hepatotoxicity. The objective of the present study was to investigate whether the increase in hepatic catalase activity after CBF pretreatment plays a role in the hepatoprotection. An irreversible catalase inhibitor, 3-amino-1, 2, 4-triazole (3-AT), was used to modulate enzyme activity. Hepatic catalase activity in mice pretreated with CBF (500mg/kg, i.p. for 10 days) was significantly inhibited by 3-AT (either 0.1 or 0.5 g/kg, i.p.). In addition, the lower dose of 3-AT (0.1 g/kg) had little effects on biliary and urinary excretion of APAP and its metabolites, which suggests that APAP metabolism was not affected by this dose of 3-AT. In toxicity studies, the mortality rate of corn oil-pretreated mice challenged with APAP (800mg/kg, i.p.) was significantly increased by 3-AT (0.1 g/kg, i.p.) given 1 h before APAP. As expected, CBF pretreatment conferred full protection against APAP-induced hepatotoxicity. The same 3-AT treatment, however, did not abolish the hepatoprotection in CBF-pretreated mice, despite the marked inhibition of hepatic catalase activity. These results indicate that elevated catalase activity in mice exposed to CBF does not appear to mediate the hepatoprotection. Our findings also point additional support for the role of reactive oxygen species in APAP-induced hepatotoxicity. (Supported by NIH grant ES10093).

1778 **ORGANIC ANION-INDUCED CHOLESTASIS PROTECTS FROM ACETAMINOPHEN (APAP) HEPATOTOXICITY IN MALE CD-1 MICE.**


Previous studies showed that administration of the non-metabolizable organic anion indocyanine green (ICG) immediately prior to a toxic dose of APAP prevents APAP-induced liver injury by decreasing bile flow (cholestasis) in mice and rats. Studies performed in rats suggest that cholestasis can play a role in this protection. Therefore, this study was conducted to determine if the cholestatic properties of this and other model organic anions is relevant to the protection against APAP liver injury previously observed in mice treated with the cholestasis drugs: bromocriptine (BCG, 30umol/kg) and rose bengal (RB, 60umol/kg) immediately prior APAP challenge (500mg/kg, i.p.). Other groups were given mice receiving the non-cholestatic drugs (DBS, 150umol/kg) amarantus (Am, 300umol/kg) prior to APAP. Controls were given vehicle only. Liver toxicity was assessed 24 h later. Hepatic necrosis was evident at 24 h in control mice receiving APAP as determined by serial dehydrogenase activity and histopathology. Pretreatment with the cholestatic drugs BCG and RB decreased the severity of hepatocellular necrosis induced by APAP. However, the administration of the non-cholestatic drugs DBS and AM did not alter APAP-induced liver damage. APAP-induced glutathione depletion was not altered by pretreatment with any of these drugs. Since cholestatic drugs were able to protect from APAP-induced liver damage while non-cholestatic drugs did not, these data indicate that cholestasis is a contributing factor to the changes in susceptibility to APAP hepatotoxicity produced by some of these cholestatic drugs. (Supported by NIH grant ES00929).

1779 **MECHANISMS OF METALLOTHIONEIN PROTECTION AGAINST ALCOHOLIC HEPATOTOXICITY: CRITICAL ROLE OF ZINC.**

Z. Zhou, X. Sun, J. C. Lambert, J. T. Saari and J. Y. Kang. *Medicine, University of Louisville, Louisville, KY.*

Metallothionein (MT) is a low molecular weight, cysteine rich (20 cysteinyl residues among 61 amino acid) protein. MT plays an important role in cellular zinc homeostasis. Our previous studies using MT-overexpressing transgenic mice demonstrated that MT is a potent cytoprotective agent against alcoholic liver injury. The present study was undertaken to determine (1) whether MT provides protection against alcoholic liver injury through zinc, and (2) whether zinc prevents alcoholic hepatotoxicity through its antioxidant property. An unique MT-knockout (MT-KO) mouse model was used. MT-KO mice along with their wild-type 129/Sv mice were assigned into four groups: control, ethanol-only, zinc-only and zinc-ethanol. Ethanol was administered by gavage at a dosage of 5g/kg every 12 h for 3 doses, and zinc sulfate was injected by i.p. at a dosage of 5 mg/kg/day for three days before ethanol treatment. Hepatic MT and zinc concentrations, liver pathological changes and oxidative stress were determined. MT-KO mice showed undetectable hepatic MT content and lower zinc concentration than wild-type mice. Zinc treatment significantly elevated hepatic MT as well as zinc concentrations in wild-type mice and only hepatic zinc concentrations in MT-KO mice. Under light and electron microscopes, pathological changes were observed in the livers treated with ethanol, while the cellular injury was much severer in the MT-KO mouse. However, this acute alcoholic hepatotoxicity was significantly inhibited by zinc in both MT-KO and wild-type mice. Furthermore, ethanol treatment decreased hepatic reduced glutathione concentration and increased hepatic lipid peroxidation, though wild-type mice showed less lipid peroxidation than MT-KO mice. This hepatic oxidative stress was abrogated by zinc pretreatment in both MT-KO and wild-type mice. These results thus demonstrate that zinc plays a critical role in MT protection against alcoholic liver injury through its antioxidant action.
1783 MECHANISMS OF POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY AND LETHALITY IN TYPE II (NIDDM) DIABETIC RATS.
S. P. Sawai1, P. B. Limaye1, K. Shankar2, T. J. Bucci2, and H. M. Mehendale2.
1Toxicology, The University of Louisville at Monroe, Monroe, LA and 2Pathology
Associate International, NCTR, Jefferson, AR.

The objective of this study was to develop a refined animal model of Type II diabetes and investigate the effects of CCl4 hepatotoxicity in Type II diabetic rats. Diabetic rats was induced in male Sprague-Dawley rats (100-124 g) maintained on high fat diet (20% fat, 24% protein, 5.4% carbohydrate) for 24 days. On day 14, streptozocin (STZ, 45 mg/kg, ip, in citrate buffer) was injected. Appetite controls i.e., high fat diet, normal diet and normal diet + STZ were maintained. On day 24, Type II diabetes was confirmed by hyperglycemia (600 ± 13 mg/dl) and normoinsulinemia before use in experiments. An ordinary non-healable dose of CCl4 (2 ml/kg, ip, 1:1 in corn oil) yielded 100% mortality in Type II diabetic rats. A time course study of 0 to 96 h was conducted after CCl4 challenge. In the diabetic rats, liver injury increased as assessed by plasma ALT, AST and histopathology. Liver function, measured by plasma bilirubin, was markedly decreased at 24 h. Liver glycogen measured as an indicator of hepatic energy status declined. Death resulted due to hepatic failure as indicated by hyperammonemia and hypoglycemia between 24 to 48 h. Hepatic microsomal CYP 2E1 measured by Western blot analysis was induced in Type II diabetic rats, about 40% above controls. Tissue repair, assessed by proliferating cell nuclear antigen (PCNA), was inhibited. In the non-diabetic rats, the release of cytokines from monocytes was increased. These findings suggest that just as Type I diabetes, Type II diabetes also is a predisposing factor for CCl4 hepatotoxicity.

1784 PROTECTIVE MECHANISMS AGAINST ACETAMINOPHEN TOXICITY IN DIABETIC MICE: ROLE OF PPAR-α ACTIVATION.
K. Shankar1, V. S. Vaidya1, E. J. Manzoori1, M. J. Ronis2, T. J. Bucci3, J. C. Corton1, and H. M. Mehendale2. 1Department of Toxicology, The University of
Louisiana at Monroe, Monroe, LA; 2Department of Pharmaceutical Sciences,
University of Connecticut, Storrs, CT; 3Arkansas Children's Hospital Research Institute, Little Rock, AR; 4Pathology Associates Inc., NCTR, Jefferson, AR and 5CHT Center for
Health, Research Triangle Park, NC.

Our recent work has demonstrated diabetic mice to exhibit significantly less liver injury and mortality together with augmented compensatory tissue repair after challenge with acetaminophen (APAP). Diabetes causes activation of PPAR-α just as peroxisome proliferators, and both protect against APAP toxicity. The present work examines the mechanistic role of PPAR-α activation in diabetic hepatotoxicity. Protective mechanism against lethal APAP challenge was ACAT: PPAR-α mice were diabetic by streptozocin (150 mg/kg, ip, citrate buffer) did not show CYP4A1 induction (a marker of PPAR-α activation), unlike wild-type (WT) diabetic phenotypes which showed considerable CYP4A1 induction. Treatment with APAP (600 mg/kg, ip in saline, a dose in WT non-diabetic mice, WT-diabetic mice showed only 30% mortality and 50% less liver injury measured by ALT and histopathology. In contrast, diabetic-PPAR-α mice did not exhibit any protection against APAP toxicity suggesting the importance of PPAR-α in diabetes-induced protection. S:Ph-PCNA synthesis and PCNA immunohistochemistry after injury showed earlier and robust tissue repair in WT-diabetic mice, but not in the PPAR-α diabetic mice. The upregulation of compensatory tissue repair in the diabetic mice appears to be mediated via PPAR-α activation. Although hepatic CYP2E1 and 1A2, which are involved in APAP bioactivation, were lower in the WT-diabetic mice, covalent binding of APAP was unchanged. Western analysis of bound 58, 36 and 44 KDA acetaminophen binding proteins was similar among diabetic and non-diabetic mice. Here we report PPAR-α activation and upregulated tissue repair repair as primary mechanisms of protection against APAP toxicity in diabetes.

1785 UPREGULATED SIGNAL TRANSDUCTION ENHANCES LIVER TISSUE REPAIR UPON TOXIC CHALLENGE IN CALORIC RESTRICTED RATS.
U. M. Apps1, P. B. Days1, M. Z. Chen1, T. J. Bucci1, A. Warnerstrom1, and H. M. Mehendale1. 1Department of Pharmaceutical Sciences, University of
Louisiana at Monroe, Monroe, LA; and 2Pathology Associates Inc., NCTR, Jefferson, AR.

We have previously reported that moderately caloric or diet restricted (DR) rats are protected from lethal hepatic toxicant challenge of thioacetamide [600 mg/kg TA; 90% lethality in Ad libitum (AL) vs. 30% in DR rats] due to timely and robust compen-
soratory liver tissue repair response. This study was designed to investigate the role of various signal transduction pathways in the stimulation of higher liver regeneration in the DR rats over a time course of 0 to 72 h after TA administration. Pro-inflammatory cytokines TNF-α and IL-6 were estimated in plasma by ELISA and in liver by either RT-PCR analysis or immunohistochemistry. Although, no significant differences in plasma TNF-α levels was observed between AL and DR, slightly higher TNF-α mRNA was found in the livers of DR rats at 48 and 72 h after TA injection. Plasma IL-6 was significantly higher in DR, which peaked at 48 h (6-fold higher than in AL). IL-6 in liver measured by immunohistochemical analysis was consistent with these results. TNF-1 and IL-6 mRNA did not change in AL or DR at any time after TA administration. Expression of growth factors TGF-β and HGF was studied by immunohistochemical analysis in liver of AL and DR rats after TA administration. DR rats exhibited higher and consistent expression of both TGF-α and HGF during the phase of acute mitogenesis while expression in AL rats was decreased and diminished. Western blot analysis of EGFR (TGF-β receptor) indicated that DR rats have higher EGFR receptor levels which remained consistent until 48 h. Similar higher c-met (HGF receptor) protein was observed in DR rats at 48 h. Taken together, these data indicate that the higher liver tissue repair is stimulated in DR rats by timely expression of the pro-mitogenic factors leading to subsequent upregulation of the signal transduction pathways involved in liver regeneration. (Supported by ES-059783)

1786 HIGHER EXPRESSION OF TNF-α AND IL-6 IN LIVER OF DIET RESTRICTED RATS AFTER THIOACETAMIDE-INDUCED LIVER INJURY.

M. Z. Chen, U. M. Appe, P. B. Limaye and H. M. Methenlade, Department of Toxicology, College of Pharmacy, The University of Louisiana at Monroe, Monroe, LA.

Previous studies in our laboratory have shown that moderate diet restriction (DR, 35% for 2 days) protects male Sprague-Dawley rats from lethal dose of thioacetamide (TA). The protection in DR rats is due to enhanced liver tissue repair. Studies indicate that pro-inflammatory cytokines, TNF-α, IL-6 and their receptors, may play an important role in stimulating quiescent liver cells to proliferate. The present studies were designed to investigate TNF-α/TNFR1 and IL-6/IL-6R expression in TA poisoned rats over a time course of 0 to 72h after injecting TA. AL and DR rats received an orally lethal dose of TA (600 mg/kg in saline, ip). Liver injury was measured by ALT, AST and histopathology, and tissue repair was measured by 5-bromo-deoxyuridine incorporation and PCNA. Plasma TNF-α and IL-6 were assayed by ELISA. Expression of mRNA in the liver was detected by semi-quantitative RT-PCR. The data indicate that TNF-α in plasma and its mRNA in liver tissue increase in both DR and AL groups after TA administration. There was no significant difference in plasma TNF-α in DR and AL rats at any time point. DR rats had significantly higher TNF-α mRNA in liver at 24 to 72h as compared to AL rats. IL-6 in plasma and its mRNA in liver also increased after TA administration. DR rats had 6-fold higher plasma IL-6 at 48h coincident with higher IL-6 mRNA in the liver. Immunohistochemical analysis of IL-6 in liver indicated localization mainly in sinusoidal endothelial cells, suggesting that these cells may be source of IL-6. In both AL and DR rats, TNFR1 and IL-6R mRNA expression was similar over the time course. Because expression of TNFR1 and IL-6R was unchanged, upregulation of pro-mitogenic signaling is achieved by up-regulated TNF-α and IL-6 in DR rats compared with AL rats. These results indicate that higher expression of TNF-α and IL-6 may play an important role in higher tissue repair in DR rats upon liver injury induced by TA. (Supported by ES-059783)

1787 EFFECTS OF TICLOPIDINE, AN ANTIPATELATE AGENT, ON RAT MODELS OF HYPERBILIRUBINEMIA.


Ticlopidine, an antipatelet agent, has been reported to cause cholestatic hepatitis in patients with rare cases. To approach the mechanism underlying the event, we investigated effects of ticlopidine on hepatic function and morphology of Eishi hyperbilirubinuric rats (EHBH), Sprague-Dawley rats (SDR), as an original strain of EHBH, and bile duct ligated rats. Repeated oral administration of ticlopidine (100 and 300 mg/kg/day for 7 consecutive days) remarkably raised serum bilirubin level only in EHBH from day 2. On day 7, the bilirubin level was 5.8 mg/dl (control 1.37 mg/dl or 7.41-11.24 mg/dl) than that of the vehicle control. This change was accompanied by slight elevations in serum bile acid, cholesterol and phospholipid levels without increased serum transaminase activities. Histopathologically, no hepatic necrosis and cholestasis were observed. In SDR and bile duct ligated rats, deterioration was neither functionally nor morphologically observed. To elucidate the mechanism of ticlopidine-induced hyperbilirubinemia in EHBH, the effects on bile flow and bilirubin and bile acid excretion into the bile were investigated. Ticlopidine did not affect these parameters, suggesting that impairment of biliary secretion did not relate to the enhanced serum bilirubin level. In the analysis of hepatic transporters mRNA levels involved in bilirubin and bile acid transport by a real time PCR, a significant increase in rat multidrug resistance-associated protein 2 (Mrp2) mRNA level as a transporter for excretion into peripheral blood was noted. Messenger RNA level of heme oxygenase-1, a rate-limiting enzyme in the catabolism of heme to bilirubin, was also elevated compared to the vehicle control (P<0.05 by Student's t test), whereas this phenomenon was not seen in SDR. These results provide the possibility that the facilitation of bilirubin formation and transport from the liver into peripheral blood through Mrp2 may contribute to ticlopidine-induced hyperbilirubinemia in EHBH.

1788 QUANTIFICATION OF HEPATOCYTE PROLIFERATION AND APOPTOSIS FOLLOWING ILEAL RESECTION IN RATS.

R. Duan, M. Grazinski, C. Wilker, B. Keller and E. Bloemme, Global Toxicology, Pharmacia, Skokie, IL. Sponsor: M. Schlesser.

Bile acids regulate the expression of various molecules involved in cholesterol and lipid metabolism by binding the nuclear receptor FXR and activating FXR-mediated transcription. Evidence also suggests that bile acids may regulate the proliferation of hepatocytes. To investigate the role of bile acids on hepatocyte proliferation and apoptosis in vivo, we used a rat model of ileal resection to reduce the ileal bile acid transporter (IBAT)-mediated enterohepatic recirculation of bile acids. Rats were sacrificed 2, 4, and 13 weeks following ileal resection. Cholesterol, "alpha-hydroxy" bile acids, FXR activity and FXR co-activator PPARα were measured to determine the decrease in ileal bile acid reabsorption. Proliferating and apoptotic hepatocytes were detected by immunohistochemistry using PCNA as a marker of cell proliferation and activated caspase-3 as a marker of apoptosis. The proliferation and apoptotic rates were quantified using computer-assisted image analysis. Our results demonstrated a slight decrease in liver weights and significant increases in Cyp7a and fecal bile acid concentrations following ileal resection. In addition, a moderate, but significant decrease in hepatocyte proliferation was observed 2 and 4 weeks after surgery, while no differences in the apoptotic rate among groups were detected at any time points. In conclusion, inhibition of ileal bile acid reabsorption through surgical ileal resection is associated with slight decreases in the rate of hepatocyte proliferation and slightly decreased liver weight, suggesting that physiologic concentrations of bile acids may regulate hepatocyte proliferation in vivo.
were provided with drinking water containing 1% betaine. DMN treatment at this dosage regimen resulted in hepatic fibrosis as determined by changes in hepatic 4-
hydroxyproline content and serum biochemical parameters, such as alanine aminotransferase, aspartate aminotransferase, total bilirubin and plasma protein
contents. Hepatic microsomal enzyme activities were also reduced in rats treated with DMN. Betaine intake significantly decreased the changes induced by DMN, which was further supported by the results of histopathological examination of rat liver. DMN treatment also elevated hepatic concentration of malondialdehyde measured by HPLC analysis, which was completely blocked by betaine. In liver of rats treated with DMN, S-adenosylmethionine level was decreased but cysteine, taurine, glutathione disulfide and homocysteine levels were all increased. Consistent changes in the activities of critical enzymes involved in the transsulfuration pathway were also observed. Betaine intake inhibited all the changes in the metabolic intermediates/products and enzyme activities induced by DMN. It is suggested that antifibrotic activity of betaine could be associated with its potential for maintaining homeostasis of hepatic sulfur-containing amino acid metabolism.

1791 ENDOThelial CELL INJURY AND FIBRIN
DEPOSITION IN RAT LIVER AFTER MONOCROTALINE
EXPOSURE.

B. L. Coppola, P. E. Gancz and R. A. Roth, Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

Monocrotaline (MCT) is a pyrrolizidine alkaloid plant toxin that produces hepato-toxicity in people and animals. Human exposure to MCT occurs through consumption of contaminated grains and herbal medicines. Intraperitoneal injection of 300 mg/kg MCT into rats produced time-dependent hepatic parenchymal cell (HPC) toxicity beginning 12 hours after treatment. To determine the development of damage to sinusoidal endothelial cells (SECs) in the liver, SEC injury was quantified using immunohistochemical staining and by increased plasma hyaluronic acid concentration. MCT produced extensive SEC injury that was restricted to centrilobular regions by 8 hours after treatment. To determine if damage to SECs in the liver stimulated activation of the coagulation system, fibrin deposition was quantified in the liver using immunohistochemistry. Extensive fibrin deposition occurred in the liver after MCT treatment and was restricted to centrilobular regions. Fibrin deposition occurred simultaneously with SEC damage and before HPC injury. Accordingly, the hypothesis was tested that activation of the coagulation system required for MCT-induced liver injury. Treatment of rats with anticoagulant heparin significantly prevented MCT-induced fibrin deposition in the liver and HPC injury. By contrast, heparin did not prevent MCT-induced damage to SECs. These results suggest that activation of the coagulation system is required for MCT-
induced HPC injury but not SEC injury in the liver. (Supported by NIH grants ES04139 and DK30728.)

1792 PROGRESSIVE HEPATOTOXICITY DUE TO A
THIAZOLIDINEDIONE CONTAINING ANALOGUE OF
N-(3, 5-DICHLOROPHENYL)SUCCINIMIDE (NDS) IN
FISCHER 344 RATS.

E. L. Kennedy, P. J. Harrison and R. Tchao, Dept. of Pharmaceutical Sciences, Univ. of the Sciences in Phila., Philadelphia, PA.

3-(3', 5'-dichlorophenyl)thiazolidine-2, 4-dione (DCPT) is an analogue of the naphthoatoxidated fungicide, N-(3, 5-dichlorophenyl)succinimide (NDS). The target organ for toxicity changed from kidney to liver when the succinimide ring of NDS was replaced by the thiazolidinedione (TZD) ring of DCPT. To further investigate DCPT-induced toxicity a time course study was conducted. Male, Fischer 344 rats were administered DCPT (0.6 mmol/kg, i.p. in corn oil), and kidney and liver function were assessed at 24, 72, and 96 hours post-dosing. DCPT treatment produced marked and persistent diuresis. Ketonuria was seen up to day 2 of the experiment. A sharp increase in urine glucose levels was observed from day 1 to day 2. However, by day 4 urine glucose values returned to day 0 levels. Also, body weight, and water and food consumption decreased on day 1, but were elevated above day 0 values by day 2 and beyond. Kidney and liver weights were not elevated throughout the experiment, however alanine aminotransferase (ALT) levels were increased at 24 hours (380.00 ± 63.77), compared to 72 (25,75 ± 4.80) and 96 (25.25 ± 3.33) hours post-dosing. The histological data support these toxicological findings because there was a focal infiltration of neutrophils from the vasa-luteo into the surrounding tissue 24 hours post-dosing. At 72 and 96 hours the neutrophils were absent, however diffuse damage was seen throughout the liver. The results suggest that DCPT has diuretic, ketogenic and glucosuria activity. Furthermore, DCPT appears to produce progressive hepatotoxicity in rats. Since liver damage was reported in patients taking clofibrate and other TZD-containing
insulin sensitizers, DCPT may be a useful model compound for studying the toxicity of these drugs. However, the role of the TZD ring in DCPT-induced hepato-toxicity requires further investigation.

1793 LACK OF SEX DIFFERENCES IN ALCOHOL-
INDUCED LIVER DAMAGE IN RATS FED LOW
CARBOHYDRATE DIETS.

M. J. Roni, T. M. Badger, R. Haksar, S. Konorur, E. Albano, M. Ingelman-Sundberg and K. O. Lindros, Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR. *Pathology, University of Arkansas for Medical Sciences, Little Rock, AR. **Medicines, University A Sogudova of East Pindemum, Novara, Italy, 1*Molecular Toxicology, Karolinska Institute, Stockholm, Sweden and 2*National Public Health Institute, Helsinki, Finland.

It has been reported that female rats are significantly more susceptible to alcohol-induced liver damage (ALD) than male, in a model of ALD which utilizes intragastric feeding (Limuto et al. Am. J. Physiol., 272:G1186-94, 1997). This has been linked to estrogen-associated increases in Kupffer cell expression of the CD14 endo-toxin receptor. We have attempted to replicate these data using two different models of ALD, one involving intragastric feeding of ethanol as part of total enteral nutrition (TEN) and one involving the use of a low carbohydrate liquid diet. Both of these models have previously been shown to produce ALD, but do not result in statistically significant increases in plasma endotoxin. Groups of N = 8 to 10 male and female Sprague-Dawley rats, 300 g were placed on the TEN system or the li-
quid diets for 42 to 60 days. Blinded pathological evaluation of liver samples was performed. In both models, ethanol treatment at 12 to 13 g/kg/d produced statistically significant liver hypertroplasia accompanied by increases in plasma ALT, steatosis, inflammation and necrosis and induction of CYP2E1 (P ≤ 0.05). However, in neither model, were females significantly more damaged than males. Total pathol-
ogy at 45 days in male ETOH males 19.6 ± 0.6, female ETOH males 8.7 ± 0.6. liquid diet model ETOH males 7.7 ± 0.7 and females 8.1 ± 0.5. Thus, our data do not support the previous conclusion that female rats have a greater susceptibility to ethanol-induced hepatotoxicity than males. Supported in part by R21 AA12031 (T.M.B).

1794 EFFECTS OF THE DIETARY ANTIOXIDANTS
BUTYLATED HYDROXYTOLUENE (BHT) AND N-
ACETYL CYSTEINE (NAC) ON ALCOHOL-INDUCED
LIVER DAMAGE IN A RAT MODEL UTILIZING TOTAL
ENTERAL NUTRITION.

R. Haksar, S. J. Roni, S. Konorur, T. Fletcher, M. Ferguson, M. Perry, S. Sharp, M. Reeves, R. Prior and T. M. Badger, Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR. *Pathology, University of Arkansas for Medical Sciences, Little Rock, AR and Arkansas Children's Nutrition Center, Little Rock, AR.

We have developed a total enteral nutrition (TEN) model to study alcohol-induced liver damage (ALD). It has been suggested that development of ALD is dependent upon free radical formation as the result of CYP2E1-dependent metabolism of ethanol and unsaturated fatty acids. In this study we examined the protective effects of two different antioxidants -- the free radical scavenger BHT and the glutathione precursor NAC. Six groups of N = 8 Sprague-Dawley rats (300 g) were used. Groups were placed on TEN, TEN + BHT (150 mg/kg/d), TEN + NAC (4.2 g/kg/d), ETOH, ETOH + BHT and ETOH + NAC for 45 d. NAC treatment significantly increased total anti-oxidant capacity of liver cytosol in TEN controls using the 12-OCacyl test (P ≤ 0.05). Ethanol treatment reduced ORAC values significantly in both the ETOH and ETOH + NAC groups (P ≤ 0.05) but the values for the ETOH + NAC group were insignificantly different from TEN controls showing that NAC consumption significantly protected the liver from oxidative stress produced by ETOH. In contrast BHT treatment reduced ORAC values in control ani-
mals but ETOH treatment had no further effect. Whereas CYP2E1 apoprotein was induced 1.6-fold in ETOH and ETOH + NAC groups, it was only induced 5-fold in the ETOH + BHT group. Significantly elevated liver weight, plasma ALT (P ≤ 0.05) and typical pathology associated with ALD (steatosis, inflammation and necrosis) were observed in all three groups of ethanol-treated rats and neither treatment provided significant protection against ethanol-associated liver damage. These data cast doubt upon the roles of CYP2E1 and free radicals in development of ALD. Supported in part by AA08645 (T.M.B).

1795 POTENTIATION OF ALLYL ALCOHOL
HEPATOTOXICITY BY ENDOTOXIN REQUIRES
ACTIVATION OF THE COAGULATION SYSTEM.

S. Kinser, R. A. Roth and P. E. Gancz, Pharmacology and Toxicology, Michigan State University, E. Lansing, MI.

Noninjurious doses of bacterial endotoxin (LPS) potentiate allyl alcohol-induced liver damage in rats. This enhanced injury could not be replicated in the isolated, buffer-perfused liver and was attenuated in vivo by heparin, suggesting that the coagulation system is required for the response. Inasmuch as heparin has effects other than anticoagulation, rats were dosed with warfarin (20 mg/kg, p.o.) or its vehicle.
42 and 18 hours before 1PS (1 x 10^4 EU/kg, i.v.). Rats were then treated two hours after LPS with allyl alcohol (30 mg/kg, i.p.). Like heparin, warfarin attenuated the increase in serum ALT activity of rats treated with LPS/allyl alcohol, suggesting that the coagulation system contributes to the toxicity of this combination. This conclusion is supported by the fact that 1PS (1 x 10^4 EU/kg, i.v.) or allyl alcohol were administered in this study to rats treated with L-ascorbic acid, which is a known antioxidant. These results indicate that rats treated with LPS/allyl alcohol may participate in mechanisms involving the coagulation cascade, and the role of the liver in the toxicity of these compounds may be more significant than previously thought.

**Activation of Protein Kinase C by Allyl Alcohol-Induced Toxicity in Rat Hepatocytes**

J. F. Maddox, N. Teshiwa, R. A. Roth and P. F. Gagey, Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

Allyl alcohol (AA)-induced toxicity in hepatocytes is well established, though the specific mechanism by which AA leads to cell death is not known. Several downstream effects of AA have been identified, including protein synthesis, cell death, and lipid peroxidation. However, early events in the process have not been identified. The purpose of this study was to examine whether specific signal transduction pathways could be associated with AA toxicity in hepatocytes. Rat hepatocytes were isolated and treated with AA for 24 hours, which induced cell death. AA-induced cell death was accompanied by the activation of MAPK and PI3K, and the inhibition of these pathways protected cells from AA-induced toxicity. The results suggest that the activation of MAPK and PI3K pathways is a key event in AA-induced toxicity, and that the inhibition of these pathways may be a potential therapeutic target for the treatment of AA-induced liver toxicity.

**Species Comparison of Vitamin K1, 2, 3-Epoxydeactase Activity**

C. R. Wilson, J. M. Sauer, G. P. Carlson, R. Wallin and S. B. Hoseney, Purdue University, West Lafayette, IL; EEl Lilly & Company, Indianapolis, IN and Wake Forest University, Winston-Salem, NC.

In the hepatic vitamin K cycle, vitamin K1, 2, 3-epoxydeactase is the microsomal enzyme responsible for catalyzing the reduction of vitamin K1, 2, 3-epoxide (VKO) to vitamin K1 hydroquinone (VKH). VKO is a potent inhibitor of posttranslational coagulation factor synthesis. The modification is essential for synthesis of biologically active factors II (prothrombin), VII, IX, X, and X, and proteins C, S, and Z of the blood coagulation system. To expand our understanding of hepatic vitamin K metabolism and function across species, a comparative study of vitamin K1, 2, 3-epoxydeactase (VKOR) activity in different species was conducted. Using an in vitro assay for VKOR activity, the enzyme kinetic constants Km and Vmax have been determined for bovine (N = 5), canine (N = 5), human (N = 1), murine (N = 5), ovine (N = 5), porcine (N = 5), and rat (N = 5) hepatic microsomes. Microsomal incubations contained 1 ml of microsomes (3 mg protein/ml), the substrate VKO (1 mM - 10 μM), and 2 mM dithiothreitol (reducing agent). The data suggest that differences in VKOR activity may be due to variations in the expression and activity of the enzyme across species. Overall, these findings provide valuable insights into the regulation and function of VKOR in different species, which may be critical for the development of therapeutic strategies targeting vitamin K metabolism.

**In Vitro Metabolism of Diethyleneglycol Monoethyl Ether (DEE) and Ethyleneglycol Monomethyl Ether (EgE) by Rat and Human Hepatocytes**

S. E. LeValley, L. Sharp, E. Hegwer and C. E. Green, Metabolism and Pharmacokinetics, SRI International, Menlo Park, CA.

Diethyleneglycol monoethyl ether (EGEE) is converted to a toxic metabolite, ethyleneglycol monomethyl ether (DEE), which is structurally similar to EGeE but its metabolism is not known. The objective of this study was to compare the in vitro metabolism of DEE and EGeE by rat and human hepatocytes to determine whether DEE is likely to be converted to toxic metabolites in vivo. Rat and human hepatocytes were isolated and suspensions were incubated with 15, 150, and 1500 μM C18-DEE or 150 μM C13-DEE. Aliquots of the incubations were removed up to 4 hr and were analyzed by HPLC with radiochemical detection. The metabolic profiles were characterized by the formation of a major metabolite, which was identified as ethyleneglycol monomethyl ether (EGEE) (96.5%) and EGeE (52.18%). Human hepatocytes did not metabolize DEE to C12-DEE to a significant extent; approximately 98 to 99% of the radioactivity remained unchanged. EGeE was readily metabolized by human hepatocytes to ethyleneglycol (7.66% and 25.37% for 2 specimens) and EGeE (17.57% and 69.50%). In summary, EGeE was readily metabolized by both rat and human hepatocytes to EGeE and ethyleneglycol, and the rat liver cells metabolized DEE to a higher rate than human liver cells in agreement with previously published in vitro metabolism data (Green et al., J. Org. Chem., 52, 1816). Human hepatocytes did not metabolize DEE significantly. No EGeE or EGeE was formed from DEE by either rat or human hepatocytes.
PROTEIN EXPRESSION CHANGES IN THE RABBIT LIVER AFTER 90-DAY JP-8 EXPOSURE.


The military jet fuel JP-8 and its commercial counterpart Jet A are used worldwide, causing exposure to liquid fuel, JP-8, and vapor. While most studies have found little toxicity from acute exposure, long-term effects are not well studied. Sprague-Dawley rats were exposed to 250, 500, or 1000 mg/kg JP-8 vapor for 6 hours per day, 7 days/week for 90 days. Livers were removed, homogenized, and frozen into crude nuclear, mitochondrial, plasma membrane, and cytosolic fractions. Proteins were identified by SDS-PAGE analysis and immunoblotting for cytokeratin P450 isozymes, apocytochrome P450, and other protein factors, and distinct changes in the expression of specific proteins identified. Proteomic data available from the same exposure groups allows a balanced interpretation of the changes in protein expression in the rat liver.

MORPHOLOGICAL CELL TRANSFORMATION OF C3H10T/FRA18(C3H8) MOUSE EMBRYO CELLS BY THE K-REGION DIHYDRODIOL OF BENZO[a]PYRENE OCCURS IN COINCIDENCE WITH DNA DAMAGE BUT WITHOUT THE FORMATION OF DETECTABLE STABLE COVALENT DNA ADDUCTS.


Benzo[a]pyrene (BaP) is the most thoroughly studied polycyclic aromatic hydrocarbon (PAH). Many mechanisms have been suggested to explain its carcinogenic activity, yet many questions still remain. K-region diols of PAHs are common metabolites and have been thought to be reactive products. Here, we present evidence that the K-region diol of BaP, diol BaP induced MCT in C3H10T/Fra18(C3H8) mouse embryo fibroblasts (C3H10T/Fra18(C3H8) cells). The diol also induced DNA damage in these cells but did not form stable covalent DNA adducts. BaP, 4, 5-diol and BaP induced MCT in C3H10T/Fra18(C3H8) cells as significant numbers of Type II and Type III foci were produced over a concentration range of 3.8-10.5 μM for BaP, 4, 5-diol and 2.1-8.1 μM for BaP. The dose-response curves for BaP, 4, 5-diol and BaP were the same. Both BaP, 4, 5-diol (8 and 10 μM) and BaP (1, 4, 8, 10 μM) induced DNA damage as determined by the comet assay after 1 hr of treatment without significant concurrent cytotoxicity. Furthermore, the distributions of comet tail contents were altered in the BaP, 4, 5-diol and BaP treatment groups compared to the controls. DNA adduct patterns in C3H10T/Fra18(C3H8) cells were examined after EP treatment using 2D-postlabelling techniques and TLC electrophoresis systems designed for polars. While BaP treatment produced one major DNA adduct identified as BaP, 4, 5-diol, no stable covalent DNA adducts were detected in the DNA of BaP, 4, 5-diol treated cells. In summary, this study provides evidence for the DNA damage and MCT activities of the K-region diol of BaP in the absence of covalent stable DNA adducts. In concert with the reported MCT activities of K-region diols of other PAHs, these data suggest a new mechanism/pathway for the bioactivation of PAHs. This abstract does not reflect EPA policy.

A ROLE FOR HEAT SHOCK PROTEIN 90 (HSP90) IN INTRACELLULAR TRAFFICKING OF BENZO[a]PYRENE (BaP) IN RAINBOW TROUT LIVER.

L. R. Curtis, T. Munsatre, C. Doneanu and D. Barfordy. EMT Oregon State University, Corvallis, OR and Chemistry, Oregon State University, Corvallis, OR.

Feeding rainbow trout 0.3 to 0.4 mg/kg/day of dieldrin for 9 to 12 weeks markedly alters the disposition of a subsequent dose of radiolabeled dieldrin and benzo[a]pyrene. After a single challenge dose of 10 μg/kg of ['H]dieldrin or ['C]BP the concentrations of their equivalents in bile increase 2 to 5-fold and 3 to 12-fold in visceral fat of dieldrin fed对照. This phenomenon has been primarily explained by an increase in binding capacity and intracellular trafficking of [PH]BP, rather than by microbial metabolism. Size exclusion chromatography on Sephadex S-300 HR of hepatic cytosol of control and dieldrin fed fish, separates a putative [H]BP binding protein aggregate that is greater than 300 kD. One component of this aggregate has been identified as HSP90 by electrospray ionization mass spectrometry. We propose that this aggregate trafficking BP to sites of metabolism or secretion in hepatocytes. In low dose in vivo experiments intracellular trafficking may be a rate limiting step for hepatic disposition of lipophilic xenobiotics.

DMBA INDUCED APOPTOSIS IN MURINE preB CELLS IS DEPENDENT ON ACTIVATION OF CASPASE-8.


Polycyclic aromatic hydrocarbons (PAHs) are known to be both carcinogenic and immunotoxic. We have demonstrated that DMBA, a prototype PAH causing apoptosis in a murine preB cell line (70Z/3) cocultured with a bone marrow stromal cell line (BMS2). We have also observed that in vivo treatment of C57Bl/6 mice with DMBA (50mg/kg) results in marked hypocellularity in the bone marrow. The aim of this study was to identify the specific apoptotic pathways activated by DMBA in vitro and in vivo. We observed caspase-8 cleavage in 70Z/3 cells following treatment with DMBA in vitro. Apoptosis was blocked by ZVAD-fmk and Z-LEHD-fmk, which are pan-caspase and caspase-8 specific inhibitors, respectively. Although a caspase-9 specific inhibitor (ZLEHD-fmk) failed to block cleavage of caspase-8, caspase-9 activation was blocked by the addition of the caspase-8 specific inhibitor (ZLEHD-fmk). These results suggest that caspase-8 activation is upstream of caspase-9 activation in preB cells undergoing apoptosis as a result of exposure to stromal cell and DMBA metabolites. Typically, caspase-8 is activated in response to binding of death receptors by their cognate ligands. DMBA induced apoptosis was not blocked by the addition of a neutralizing mAbs to FasL, indicating that the Fas/FasL system was not responsible for caspase-8 activation in our system. We have detected up-regulation of Protein Kinase R (PKR), a double stranded RNA dependent kinase that is capable of death receptor independent activation of caspase-8 in response to DMBA treatment. Caspase-8 activation has also been detected in the bone marrow of mice treated in vivo with DMBA, which may explain in part the hypocellularity that occurs in the bone marrow. By comparing our in vitro and in vivo systems we are able to better understand how caspase activation might lead to leukocyte apoptosis and the immunotoxic effects of DMBA.

POLYCYCLIC AROMATIC HYDROCARBON MIXTURES: ASSESSMENT OF HUMAN HEALTH RISKS AND RESEARCH NEEDS.


The Integrated Risk Information System (IRIS) Program has initiated a health assessment for chronic environmental exposure to polycyclic aromatic hydrocarbons (PAHs) mixtures. Current health assessments and guidance that are used by EPA for estimating risks from exposure to PAH mixtures were developed in the early 1990s and include 1) IRIS health assessment for 15 individual PAHs, and 2) Provisional Guidance for Quantitative Risk Assessment of PAH (1993) which provides relative potency factors (RPF) for seven PAH identified as probable human carcinogens using BENZ0A[PYRENE] (BaP) as a standard. Risk assessment of PAH mixtures has been hindered by a lack of information on the composition of mixtures, the toxicity of specific mixtures, the components that contribute most to toxicity, and the interactions and differences in mode of action of toxicity between components. This information is critical for the evaluation of risks for specific mixtures and the use of surrogate to estimate risk. More recent studies have emerged which, at least in part, address these issues; but the studies also point to data gaps and additional research needs. The status of the health assessment for PAH mixtures and a compilation of the identified research needs is presented which includes the following as exemplary data gaps: 1) oral and inhalation toxicity of various PAH mixtures and inhalation toxicity of BaP using standard chronic bioassays is lacking; 2) the use of BaP as a standard for assessing the toxicity of PAH mixtures is now questioned, but animal bioassays for potential surrogates (e.g., superfecarcinogens) and comparative in vitro studies of these PAH with other known components of PAH mixtures are minimal; and 3) although recent studies have provided more data regarding interactive effects among PAH, they are limited in scope with regards to PAH mixtures as a whole and the quantitative aspects have not been elucidated. (The opinions expressed are those of the author and should not be construed as EPA policy).

POLYCHLORINATED BIPHENYLS-INDUCED APOPTOSIS OF MURINE SPLEEN CELLS IS ARYL HYDROCARBON RECEPTOR-DEPENDENT BUT CASPASE-DEPENDENT.

Y. J. Jeon, S. H. Lee, J. Suh, Y. J. Na and H. M. Kim. College of Pharmacy, Chosun University School of Medicine, Wandojeong, South Korea. Research Institute of Biostatistics, Tarung, South Korea and Biological Sciences, Korea Advanced Institute of Science and Technology, Tarung, South Korea.

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, and many of the toxic effects including immunotoxicity are mediated by the activation of aryl hydrocarbon receptor (AhR). We previously reported that Aroclor 1254, one
of the most widely used PCB mixtures, increased DNA fragmentation in mouse spleen cells, assuming that apoptosis was correlated with the immunotoxicity of PCB (Yoo et al., 1997). In the present study we investigated the mechanism by which PCB induces apoptosis and the involvement of AHR in PCB-mediated apoptosis in mouse spleen cells. PCB 128-induced DNA fragmentation without AHR and the apoptosis was not affected by a nA-R, a well-known antagonist of AHR. PCB congeners (PCB 47, 52, 128, and 153) that have little affinity for AHR induced DNA fragmentation, whereas congeners (PCB 77, 126, and 169) that have high affinity for AHR did not induce the fragmentation. Discs from AHR congener (PCB 153) and Aroclor 1254 induced DNA fragmentation in spleen cells of both AHR knock-out mice (AHR-) and Ah low-responsive mice (DBA/2), whereas non-ortho form of PCB congener (PCB 126) did not induce the DNA fragmentation. In light of these findings, it is clear that AHR is not involved in the PCB-mediated apoptosis. PCB 153 significantly increased caspase-3 activity in both spleen cells and human leukemia cells in induced DNA fragmentation in splenic cells of both AHR knock-out mice (AHR-) and Ah low-responsive mice (DBA/2), whereas non-ortho form of PCB congener (PCB 126) did not induce the DNA fragmentation. Based on our findings, the most likely mechanism that can account for this biological effect involves the induction of caspase-dependent apoptotic cell death.

1806 EFFECTS ON PROLIFERATION, DIFFERENTIATION, AND GENE EXPRESSION OF BENZO(A)PYRENE, ARSENIC, AND CALCIUM ON NORMAL HUMAN EPIDERMAL KERATINOCYTES.

D. S. Perez, R. S. Yang and J. A. Campain. Center for Environmental Toxicology & Technology, Environmental Health, Colorado State University, Fort Collins, CO.

We are interested in developing accurate risk assessment strategies for environmentally relevant chemical mixtures. To this end, normal human epidermal keratinocytes (NHEK) were chosen as an in vitro model for measuring the carcinogenic potential of petroleum-based hydrocarbons. Our initial studies examined the effects of BENZO(A)PYRENE (BaP), on proliferation and differentiation in NHEK; perturbations in these cellular processes have been strongly linked to carcinogenesis in keratinocytes. We have demonstrated that BaP inhibits terminal differentiation in NHEK, as measured by transduced envelope (CE) formation, by up to 5.8-fold in control cells and 1.8-fold in calcium (Ca) induced cells. In comparison, arsenic (As), a known inhibitor of this process, decreased CE formation by approximately 20-fold in control cells and 3.2-fold in Ca induced NHEK. We are interested in delineating the effects of BaP on both terminal growth arrest and subsequent expression of protein markers of keratinocyte differentiation. To this end, flow cytometric analysis is being used to measure cell cycle distribution in BaP- and As-treated versus control populations and populations undergoing Ca-stimulated differentiation. BaP at 2µM has been shown to increase proliferation rates by 1.5-fold. In contrast, As at 1µM inhibited proliferation 1.6-fold; similar growth arrest is observed (1.4-fold) in NHEK treated with 2µM Ca. Our findings suggest that, although both BaP and As inhibit CE formation in NHEK, different mechanisms may be involved. Current studies will attempt to identify, through microarray analysis, molecular markers involved in the observed chemical effects. Our aim is to correlate alterations in these molecular markers with the transformation process in NHEK, and to develop predictive capacities for risk assessment of petroleum-based hydrocarbons. This research was supported by NIEHS Grant # R01 ES09655 & Minority Supplement # R01 ES09655-01S1.

1807 SUPPRESSION OF OVARIAN FUNCTION BY INHALED BENZO(A)PYRENE.


This study was conducted to evaluate the effect of BaP on ovarian function. Adult female Fisher-344 rats were randomly assigned to a treatment and a control group per exposure regimen. Two treatment regimens utilized in this study consisted of subacute exposure of rats to BaP inhalation at 0.05 or 0.1 mg/m3 of BaP carbon black aerosol, four hours a day for 10 days. From the fourth day of exposure, animals in the control (unexposed) and treatment groups were synchronized with subsequential progestereon (P2) injections (1 mg P2/animal/day for 4 days). Folliculogenesis was induced in these animals with intra-peritoneal (IP) injection of 15 IU of equine gonadotropin (EQ) about 24 hours after the last P2 injection [day 7 of BaP treatment]. Ovulatory response was subsequently induced at 48 hours post EQ by an IP injection of 15 IU of human chorionic gonadotropin (hCG; day 9 of BaP treatment). Twenty four hours post ovulation, the animals were sacrificed, ovaries collected, weighed and number of ovaries, follicles, corpora lutea and corpora albicantia counted. All rats were identified, their ovaries were weighed and corpora lutea were counted. The number of corpora lutea on both ovaries was determined by counting the total number of albumen body on both ovaries. All rats responded positively to the synchronization and folliculogenesis/ovulation induction protocol. Both regimens of BaP suppressed ovulation rates (P<0.05) compared with controls (P<0.05 mg BaP/m3, 11.0 ± 2.4 vs 24.6 ± 1.6; 0.1 mg BaP/m3, 18.7 ± 1.5 vs control, 20.9 ± 1.3) The percentage suppression of ovulation rate by BaP was exposure concentration-dependent (P<0.05). However, regardless of BaP-exposure concentration, ovaries were intact. These data suggest that (a) BaP perturbs the ability of fixed sexogenous gonadotropins to adequately initiate folliculogenesis and ovulation and (b) BaP probably acts to reduce ovulatory response to gonadotropins via a reduction in follicle-stimulating hormone (FSH) (Supported by NHF grant US01ATU39894).

1808 ROLE OF BENZ(A)PYRENE (BaP) METABOLISM IN LUTEOTROPIC ACTIVITY AND FETAL SURVIVAL OF TIMED PREGNANT RATS EXPOSED TO BaP: CARBON BLACK AEROSOL.


This study was undertaken to assess the effect of BaP metabolites on luteal maintenance and fetal survival in timed-pregnant Fisher 344 rats. Treatment consisted of subcutaneous-only inhalation exposure of rats to 25, 75, and 100 µg/m3 of BaP: carbon black aerosol, for 4 hours a day for 10 days, beginning on day 10 of gestation. Control animals were either sham-exposed to carbon black (CB) or unexposed (UNC). Blood samples were collected on days 1, 15, and 17, and 20 of gestation via ocular puncture for plasma. Number of pups per litter was determined postpartum. Luteotrophic factor in plasma samples was determined by measuring prolactin (PRL) levels by RIA. Metabolite levels in plasma and tissue samples from pregnant rats, and pups (postnatal day 1) were measured and identified by reverse phase HPLC coupled with fluorescence detection. Fetal survival declined (P<0.05) in a concentration-dependent manner. Fetal luteotrophic factor means were highest in rats exposed to BaP (25 µg/m3: 73%; 75 µg/m3: 38%; 100 µg/m3: 31%) compared with CB (77%) and UNC (86%). Plasma PRL concentrations in BaP treated rats showed a concentration-dependent decline relative to controls. The metabolite levels in plasma, uterine tissues, and pups were also exposure concentration-dependent. These results strongly suggest that the exposure-concentration-dependent disposition of inhaled BaP metabolites, especially the trans-7,8-dihydochloranthrene contributed to a suppression in luteotropic activity and reduced embryonic survival (Supported by NIEHS grant US01ATU39894, NIH grant G12RR03852, SCOR project 2506GCM08037-28, and RMC project RR/A1 30027).

1809 DIFFERENTIAL EFFECTS OF POLYCHLORINATED BI PHENYLS (PCBs) ON EXPRESSION OF HEPATIC ANION TRANSPORTERS oatp2 and oatp3 and MULTIDRUG RESISTANCE PROTEIN 2 (Mrp2) IN RATS.

L. A. Martin, M. A. Gallo, K. R. Reuhl and C. D. Klaassen. "JGPT, Rutgers University and UMDNJ, Piscataway, NJ and University of Kansas Medical Center, Kansas City, KS.

Transfer of thyroxine (T4) across plasma membranes is accomplished, in part, via transport proteins. Two proteins possibly involved in this process are organic anion transporting polypeptides oatp2 and oatp3. Additionally, Mrp2, a member of the superfamily of ATP binding cassette transporters, is implicated in the excretion of T4 glucuronide (T4-G) in bile. The purpose of this study was to determine whether PCBs, which markedly reduce serum T4, might do so by enhancing the hepatic uptake of T4, and subsequent excretion into bile, via increases in the expression of these proteins. Male rats were administered Arorcol 1254 or Aroclor 1242 for 7 consecutive days and 24 hrs after the last dose, T4 and T3 were administered iv. At 3, 5, 10, 15 and 30 min post injection, blood was collected. The presence of serum and liver was quantified by gamma spectroscopy, and transporter protein levels in liver were determined by western-blot analysis. Within one min post-injection, Aroucolon 1254 produced a 56% decrease in serum T4 and Aroclor 1242 a 30% reduction within 3 min, as compared to control. At the one min time point, Aroclor 1254 and Aroclor 1242 produced a 109% and a 54% increase, respectively, in the uptake of [3H]-T4 into liver, as compared to the control value. Oatp2 protein levels were suppressed 87% by Aroclor 1254, compared to the control, but Aroclor 1242 had no significant effect on oatp2. No significant changes in oatp3 protein levels were observed. In contrast, Arorcol 1254 and Aroclor 1242 produced significant increases in Mrp2 protein levels of 388% and 29%, respectively, relative to control. These data suggest that the increase in hepatic uptake of T4, in rats exposed to PCBs cannot be explained by upregulation of oatp2 and oatp3. However, upregulation of Mrp2 may contribute to increased biliary excretion of T4. (Supported by NIH grants ES-07146, ES-05022 and ES-08156.)
1810 MODULATION OF ACUTE NEUROBEHAVIORAL TOXICITY OF BENZYL/PROPYRENE BY METABOLISM, ANTIOXIDANT ENZYMES, AND MICROCHEMICAL PARAMETERS IN F-344 RATS.

C. R. Saunders¹, S. Mulherjee², S. K. Das³, A. Ramesh⁴ and D. C. Shockley⁵.
¹Pharmacology, Meharry Medical College, Nashville, TN and ²Biochemistry, Meharry Medical College, Nashville, TN.

The objective of this study was to understand the effects of Benzyl/propyrene (BaP) on the performance of nervous system by correlating behavioral changes with metabolism, antioxidant enzyme activities, and neurochemical parameters in selected brain regions. The neurobehavioral effects of single oral doses (12.5-200 mg/kg) of BaP on motor activity and the functional observational battery (FOB) were studied in male F-344 rats. Immediately after dosing, animals were returned to Digicam activity cages and monitored for locomotor activity, beginning at 1800 hrs at 2 hrs and for a total of 12 hrs for five consecutive days. The FOB was conducted on days 2, 12, 24, 48, 72 and 96 hrs post BaP treatment. Benzyl/propyrene parent compound and metabolites in plasma and brain tissues were measured by reverse phase HPLC. The effects of BaP on dopamine levels in the striatum were examined at 24 and 48 hrs post treatment. Control groups of antioxidant enzymes (catalase, superoxide dismutase, SOD, and glutathione peroxidase) were determined at 6 and 96 hrs in both the striatum and hippocampus of BaP exposed rats. Locomotor activity was suppressed on day 2 with recovery to control levels by day 3 in treated rats. Behavioral effects reached a maximum at 6 hrs in all FOB test measures but were reversible after 72 hrs in all dose groups. Bioavailability data suggest a strong link between BaP and the onset of behavioral effects. A dose-dependent effect was observed. Striatal dopamine content was correspondingly reduced in treated rats. Benzyl/propyrene produced a 30-50% and 12-25% upregulation of SOD at 6 and 96 hrs post treatment respectively. These findings suggest BaP cause acute neurobehavioral toxicity and modulation of striatal dopamine levels. Furthermore, the alteration of free radical status may contribute to BaP-induced neurotoxicity (Supported by MHPP/ATSDR U50ATU398048, NHLBI T32-HL07809, US Army DAMD-17-99-1-9550 and NIH 2506-GM-08037).

1811 REPRODUCTIVE PERFORMANCE OF TWO GENERATIONS OF RATS EXPOSED TO LOW LEVELS OF TECHNICAL CHLOROANE.

M. M. Mansouri¹, V. F. St. Omer², B. C. Dutri², K. M. Mizingo³, E. O. Abdulla¹, H. O. Goyal³, F. O. Johnson⁴, R. D. Mashal⁵ and K. Ali⁶.
¹Biomedical Sciences, Tuskegee University, Tuskegee, AL; ²Pharmacology, University of Health Sciences, Kano, Kano, Nigeria; ³Veterinary Physiology, Sokoni University of Agriculture, Sokoni, Tanzania. Sponsor: R. Daliq.

Technical chloroane is a persistent organochlorine mixture of four main isomers (heptachlor, cis-dichlorane, trans-chloroane, and trans-nonachlor). The effects of chloroane at environmental levels on reproductive performance of humans and other mammals are relatively unknown. Sprague-Dawley rats (P0, F1 & F2) were used in a two-generation study to evaluate technical chloroane effects on fertility index, pregnancy outcome, indices of puberty, reproductive organs weight and morphology, vaginal cytology and testicular parameters. Chloroane doses were 0.0, 0.125, 0.250, 1.0 mg/kg body weight/day. Results of the first of two replicates indicated that, chloroane delayed (P<0.05) the onset of vaginal opening in F1 females, but time to first estrus was unaffected; in contrast, F2 generation manifested no effect in time of vaginal opening, but time to first estrus was delayed at 1.0 mg/kg level. Estrus cyclicity was not affected. Time of prepubertal separation was advanced in F1 males at 0.125 mg/kg/day. Relative to the control, mating index was decreased in F1 chloroane treated groups, but not in F0. There was no treatment effect on total live litter, litter size and sex ratio in both F1 and F2 generations. At necropsy, chloroane increased uterine weight in F0 dams at 0.125 mg/kg level and reduced uterine weight of F2 males at 0.250 mg/kg level. Measurement of the seminiferous tubules revealed an increase in its diameter and epithelial height of F1 & F2 chloroane treated groups. Sperm cells motility, velocity, morphology and concentration as well as plasma testosterone levels were unaffected. These results indicated that low levels of technical chloroane disrupted the normal morphology and physiology of the reproductive organs. (Supported by: MHPP/ATSDR U50/ATU3922660088)

1812 REPRODUCTIVE TOXICITY ASSESSMENT OF CHRONIC ORAL ADMINISTRATION OF FLUTAMIDE IN MALE RATS.

A. S. Faqui¹, D. Land², W. D. Johnson³, R. L. Morrissey⁴ and D. L. McCormick⁵.
¹ITT Research Institute, Chicago, IL and ²Pathology Association International, Chicago, IL.

Flutamide [2-methyl-N-(4-nitro-3-(trifluoromethyl)phenyl)propanamide] is a non-steroidal androgen antagonist used as a therapeutic agent for prostate cancer. We have recently demonstrated that chronic dietary administration of flutamide also inhibits prostate carcinogenesis in a rat model. The present study was performed to determine the effects of a chemopreventive flutamide regimen on male reproductive parameters. Groups of 10 male Wistar-Unilever rats (9-10 weeks of age) were fed a basal chow diet or basal diet supplemented with flutamide (25 or 100 mg/kg diet) for 12 months. In comparison to diet controls, rats exposed to flutamide demonstrated significant reductions in mean testes and epididymis weights. A non-monotonic dose-response was seen: testicular atrophy was present in 10/10 rats in the low dose flutamide group, but only in 4/10 rats in the high dose group. Similarly, although both doses of flutamide reduced spermaticus number and daily sperm production, the low dose had quantitatively greater effects. Epididymal sperm obtained from flutamide-treated rats demonstrated relatively fewer sperm; greater reductions in epididymal sperm count were seen at the low flutamide dose than at the high dose. Percent abnormal sperm (kinely tail, absence of head) was increased only in samples obtained from rats receiving the low dose of flutamide; percent abnormal sperms in the high dose group was comparable to that in diet controls. Testicular histopathology was assessed in the flutamide-treated rats. Seminiferous tubules populated primarily by spermatogonia and spermatocytes relatively few spermatids were present. These data suggest that flutamide doses that confer significant protection against prostate cancer induction are likely to have significant adverse effects on male fertility. Decisions regarding the possible use of flutamide in prostate cancer prevention must include considerations of the impact of this agent on sexual potency.

1813 TRANSGENIC RATS AS A MODEL FOR THE INVESTIGATION OF ESTROGENIC EFFECTS.


Endocrine disruptors are environmental chemicals which may disrupt reproductive development. The ability xenobiotics to bind to the estrogen receptor (ER) has been used as a definition of estrogenicity. Data suggest that estrogenic disruption effects are due to the interaction of the xenobiotic with ER that regulate gene expression. Estrogenic chemicals typically bind the ER, the ER-ligand complex interacts with estrogen responsive elements (ERE), and transcription factors are recruited to the ER-ligand/ERE complex, resulting in enhanced gene expression. Receptor/ligand binding causes changes in gene expression leading to altered function of endocrine-regulated physiological systems. The initial screening methods used to detect estrogenic/aromatic estrogenic activity are combinations of ER binding activity and transcriptional cellular assay systems in vitro. The interpretation of these data in the context of the in vivo situation can be difficult. The purpose of our study was to establish a transgenic animal approach, which allows investigation transcriptional changes with functional alterations in the same animal. For this purpose, we investigated ERE-containing promoter-reporter usage in vivo. Reporter assays revealed dose dependent response curves after treatment of the MCF-7 cells with a series of 17beta-estradiol and Bapreml A concentrations. After in vitro testing, we established different transgenic rat strains on a Sprague-Dawley background using a p52 promoter-construct. Matting of the founder generation with wild type Sprague-Dawley rats revealed transgenic positive rats. RT-PCR analysis of mRNA revealed reporter-gene expression in different organs (eg. uterine) of untreated rats from different transgenic rats. Here we established a transgenic ER-receptor report rat as a model for testing estrogenicity of endocrine disruptors in vivo. Using this transgenic rat strain we have the possibility to study the effects estrogens and xenoestrogens on endocrine-regulated physiological systems.

1814 IDENTIFICATION OF A CONSUMER PRODUCT ADDITIVE THAT INHIBITS OVULATORY FUNCTIONING IN PICOMOLAR DOSES.

K. Rives and P. Talbot. Cell Biology and Neuroscience, University of California, Riverside, CA.

Our past studies have shown that chemicals in cigarette smoke inhibit ovulatory functioning in vivo and in vitro (Magers, Reprod Toxicol 1995, 9:513; Knoll, Reprod Toxicol 1998, 12:57). The purposes of this study were to identify the individual tobacco in cigarette smoke samples made from 2R1 research brand cigarettes (University of Kentucky) that inhibit oocyte pickup rate, citrate bear frequency, and infundibular smooth muscle contraction, and to determine their effective doses. Solid phase extraction and gas chromatography-mass spectrometry (GC-MS) were used to identify individual chemicals in the mainstream and sidestream cigarette smoke samples. Pyridine derivatives were the most abundant class of compounds identified by GC-MS. Commercially available pyridines were purchased, assayed for purity, and tested in dose-response studies on the oviduct using an in situ bioassay. The no observable adverse effect level (NOAEL) and lowest observable adverse effect level (LOAEL) were determined for each pyridine compound using the oocyte pickup rate, citrate bear frequency, and infundibular mus-
1815
Lack of Reproductive Toxicity in a 2-Generation Study of DEHT
Ethylenehexylterephthalate (DEHT), DOTP) in Rats

W. D. Faber, D. L. Stump, R. M. Dougall, and K. R. Miller, Wilmar Faber Toxicology Consulting, Victoria, NY; Willmar Laboratories, Attleboro, MA; Eastern Kodak Company, Rochester, NY; and Eastman Chemical Company, Kingsport, NC.

A two-generation reproductive toxicity study of DEHT was conducted in which groups of 30 male and 30 female Sprague-Dawley rats were exposed to 0, 0.3, 0.6, or 1.0% DEHT in the diet. The F0 animals received the diets for at least 30 days prior to mating and until termination of the generation; the F1 generation received diets beginning on PND 21. All females were allowed to deliver and rear their pups to weaning (lactation day 21). Mean maternal body weights and body weight gains were reduced for F0 and F1 females in the 1.0% group throughout gestation. Mean maternal body weights were also reduced for the F0 and F1 females in this group throughout lactation. Overt toxicity and mortality were seen in the 1.0% F0 and F1 groups. Increases at autopsy of the heart and liver weights (females in both generations) were observed for the 0.6% and 1.0% groups. No test article-related microscopic findings were observed in any of the tissues examined for either sex in either generation. Reproductive parameters (fertility, mating, days between pairing and estrus, gestation, parturition, and estrous cycles) were unaffected by treatment at any level during the F0 and F1 generations. Mean litter sizes, numbers of pups born, percentages of males per litter at birth and postnatal survival were unaffected by parental treatment at all concentrations. F1 and F2 offspring weights in the 0.6 and 1.0% groups were reduced throughout the pre-weaning period for the F1 generation and beginning on PND 4 and 7 for the F2 generation in the 0.6 and 1.0% groups, respectively. The NOAEL for reproductive toxicity was considered to be 1.0%. The NOAELs for parental toxicity and neonatal toxicity were considered to be 0.3%. These data complement the information from Gray et al. (2000) that indicate that DEHT does not produce an adverse effect.

1816
Effects of Prenatal Testosterone Propionate and Vinclozolin on Perinatal and Infantile Development of Male and Female Rats

C. J. Wolf, J. Furr, G. A. LeBlanc, and L. E. Gray, RTD, USEPA, Research Triangle Park, NC, and Environmental and Molecular Toxicology, NCSU, Raleigh, NC.

Prenatal exposure to both androgenic and antiandrogenic chemicals can adversely affect pregnancy and growth of offspring, but have opposite effects on sexual development. We conducted a study to determine whether an antiandrogenic chemical, vinclozolin (V), and an androgenic chemical, testosterone propionate (TP), in combination or alone, have similar or opposing effects on perinatal health and reproductive development. Sprague-Dawley rat dams were dosed on gestational day (GD) 14 (GD 1 day of plug) to GD 19 with either corn oil (vehicle, orally, V: 200 mg/kg, orally), TP (1 mg/kg, sc injection), or V + TP. Weight gain during the pregnancy period was reduced by TP (53.4 ± 9.3 g; p < 0.005) and by V (28.1 g; p < 0.005) but was further reduced by V + TP (21.9 g; p < 0.005). Live litter size on postnatal day (PND) 2 was reduced by V + TP only (5.6, p < 0.001 vs 13.8 in controls), although mean number of uterine implantation sites was not affected. Anogenital distance (AGD) in females on PND 2 was increased from control values (1.7 mm) to TP (2.6 mm; p < 0.0001) and by V (2.18; p < 0.0001). Live litter size on postnatal day (PND) 2 was reduced by V + TP only (5.6, p < 0.001 vs 13.8 in controls), although mean number of uterine implantation sites was not affected. Anogenital distance (AGD) in females on PND 2 was increased from control values (1.7 mm) to TP (2.6 mm; p < 0.0001) and by V (2.18; p < 0.0001). Mean litter size on postnatal day (PND) 2 was reduced by V + TP only (5.6, p < 0.001 vs 13.8 in controls), although mean number of uterine implantation sites was not affected. Anogenital distance (AGD) in females on PND 2 was increased from control values (1.7 mm) to TP (2.6 mm; p < 0.0001) and by V (2.18; p < 0.0001). Live litter size on postnatal day (PND) 2 was reduced by V + TP only (5.6, p < 0.001 vs 13.8 in controls), although mean number of uterine implantation sites was not affected. Anogenital distance (AGD) in females on PND 2 was increased from control values (1.7 mm) to TP (2.6 mm; p < 0.0001) and by V (2.18; p < 0.0001).
ABBREVIATED ONE-GENERATION STUDY OF DIETARY BISPHENOL A (BPA) IN CD-1 MICE.

Research Triangle Institute, Research Triangle Park, NC, Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, Toxicology, Bayer AG, Wuppertal, Germany, and Global Toxicology, General Electric Co., Pittsfield, MA.

This study duplicated the species, strain, route, and top two dietary BPA concentrations (5000 and 10,000 ppm) employed in a previous RABC study (Reel et al., 1985), but with shorter duration, one breeding, and enhanced evaluation of parental systemic/reproductive toxicity to provide the context for previously reported F1 offspring effects at these doses. F0 mice (20/sex/group) were exposed ad lib to BPA for 2-wk prebreed, 1-wk mating (F0 males necropsied after mating; 3-wk total exposure) and ca. 3-wk gestation. F0 dams and F1 littermates were necropsied at parturition (post 0). Parental systemic toxicity was present at both BPA doses: increased liver and kidney weights (WT) in both sexes; for F0 dams: reduced body weights (BW), weight gain, and feed efficiency during pregnancy; prolonged gestation (no effects on precoital interval or ovulation or uterine WT); histopathology in liver (dose-related lipocellular hypertrophy) and kidneys (dose-related tubular epithelial degeneration, necrosis and regeneration); elevated serum BUN at 10,000 ppm and reduced serum Na, K, and Cl at 5000 ppm (consistent with renal toxicity). F1 offspring toxicity was observed only at 10,000 ppm: slightly reduced urate and live pup/ litter, with no effects on pre/postimplantation loss or pup BW. This study resulted in comparable daily BPA intakes (for both sexes and doses) and confirmed litter size effects at 10,000 ppm by Reel. It also demonstrated parental systemic toxicity at both doses. Our data support the interpretation of litter size effects at 10,000 ppm as caused by the compromised status of dams (after 5-6 wks total exposure), while the lesser systemic toxicity at 5000 ppm did not cause litter size effects. F1 litter size effects at 5000 ppm were observed only by Reel only for 4th-5th litters after much longer exposure duration (>90 days).

ALTERATIONS IN THE RAT ESTROUS CYCLE BY DIBROMOACETIC ACID (DBA). RELATIONSHIP TO A SUPPRESSION IN ESTRADIOL (E2) METABOLISM.


Of the large variety of chemicals formed by chlorination of public drinking water, a number are suspected to cause reproductive alterations in humans and test animals. Members of one class, the haloacetic acids (of which DBA is a member) have been reported to alter rat sperm production & gonadal hormonal activity at elevated doses. In females, DBA was found to induce a persistent alteration in estrous cyclicity, something found in the present study using 14C exposures (0-270 mg/kg, po). Body weights were unaffected. While a fall in progesterone (seen in vitro) could impact cyclicity, it may be that E2 levels are also altered. In vitro data did not show shifts in estrous E2 release, but an impact on E2 metabolism could affect blood levels. Consequently, cyclicity (measured by ovulation rates) were tested for 4 wks with DBA (0, 10, 30, 90, 270 mg/kg in water, pH adj. to 6.8). After 8d (during which cycles continued), females were ovarcietomized & 3d thereafter implanted with estradiol capsules (OvX-E2). Daily tail nick blood was then taken for 3d. Non-E2 capsules were also implanted & blood taken (0 & 270 mg/kg DBA). Additional OvX-E2 rats (270 mg/kg) received phenobarbital (PB, 0.1% in drinking H2O) for 4d before sampling to stimulate E2 metabolism. After capsule implantation, an initial serum E2 rise then fell over the next 2d. By 72h, DBA caused a dose-related increase in E2 that at the highest dose was approx. 2 1/2-fold above controls. PB reduced DBA-elicited E2 levels. Data suggest that alterations in cyclicity seen following DBA exposure could be at least partially due to a suppression in hepatic E2 metabolism. The rise in E2 is reversible & speaks against suicide inhibition of the relevant P450 activity. The data indicate that DBA given at these doses over 2 wks acts as an endocrine disrupting chemical. While it is conceivable that lower doses over more extended periods could have similar effects on reproductive functioning, such studies remain to be conducted. (This abstract does not reflect EPA policy).

EFFECTS OF METHOXYCHLOR (M) OR ITS ACTIVE METABOLITES 2, 2, 2-BIS (P-HYDROXYPHENYL) 1, 1, 1-TRICHLOROETHANE (HTPE), ON TESTOSTERONE (T) FORMATION BY CULTURED ADULT RAT LEYDIG CELLS (LC).

E. P. Munson and R. C. Derk, Pathology and Physiology Research Branch, CDC/NIOSH, Morgantown, WV Sponsor: V. Castranova.

M is a pesticide developed as a replacement for dichlorodiphenyltrichloroethane (DDT). Its active metabolite is reported to be HTPE. Both M and HTPE have been shown to exhibit weak estrogenic or antiandrogenic activities in various in vivo or in vitro testing protocols. In the present studies, we examined the direct effects of M or HTPE on T biosynthesis by cultured LC from young adult rats. Highest concentrations of M or HTPE (1-1000 nM) caused a progressive decline in both basal and 10 nM human chorionic gonadotropin (hCG)- or 1 nM 8-Bradenanized T following exposure for 4 or 24 h, beginning at 100 or 500 nM, although the declines with HTPE generally were greater. To localize the site(s) of action of HTPE, LC were exposed to HTPE (1-1000 nM) alone for 24 h, then fresh media containing steroid precursors of T were added to follow the ability of exposed cells to convert these substrates to T over 4 h. The conversion of 0.01 mM pregnenolone, progesterone or androstenedione to T was affected by prior exposure to HTPE; however, there was a progressive decline in the conversion of the 0.01 mM 22(R)-hydroxycholesterol to T, suggesting that among the enzymes involved in the conversion of cholesterol to T, P450 cholesterol side cleavage activity is inhibited by HTPE. The concomitant inclusion of the pure estrogen antagonist, ICI 182,780, did not alter the above mentioned effects of HTPE, suggesting that the actions of HTPE are not mediated by binding to estrogen receptor alpha or beta. Also of interest, the addition of the native estrogens, 17beta-estradiol, or the antiandrogens, cyproterone acetate (1-1000 nM) or hydroxyflutamide (1-1000 nM), had no effect on basal or hCG-stimulated T following exposure for 24 h, suggesting that the actions of HTPE are not due to its estrogenic or antiandrogenic properties.

EXPRESSION OF BIOTRANSFORMATION ENZYMES IN OVARIAN INTERSTITIAL CELLS: EFFECT OF DOING WITH 4-VINYLCYCLOHEXENE AND ITS DIEPOXY METABOLITE IN MICE.

Pharmaceutical/Toxicology, University of Arizona, Tucson, AZ, Physiology, University of Arizona, Tucson, AZ, and Biological Science, Northern Arizona University, Flagstaff, AZ.

Our laboratory is investigating the role of ovarian metabolism in 4-vinylcyclohexene-induced ovotoxicity in mice. This study was designed to evaluate the effect of repeated dosing with the ovotoxicants 4-vinylcyclohexene (VCH) and its diepoxy metabolite (VCD) on expression of several bio transformation enzymes in the ovarian interstitium, whose function is poorly understood. Female B6.CF1 mice (28 wks) were dosed daily (15d) with vehicle (Con), VCH (800 mg/kg) or VCD (80 mg/kg). Ovaries were removed and fixed for immunochistology, or isolated into interstitial cells. We evaluated the effects of VCH/VCD dosing on mRNA expression and protein distribution of cyp 2c1, cyp 2a5/6, and microsomal epoxide hydrolase (mEH) using real-time PCR or confocal microscopy, respectively. Luxaturing hormone receptor was used as a marker for confocal visualization of interstitial cells. mRNA encoding cyp 2c1, cyp 2a5/6, and mEH was present in interstitial cells from control mice. However, after repeated dosing, expression was significantly decreased only for mEH (60-83% of Con, VCH, p<0.05), compared to control. Intense immunostaining for cyp 2c1, cyp 2a5/6, and mEH proteins were seen in interstitial cells from control mice. VCH dosing decreased staining intensity for cyp 2c1 (19±2.4% of Con VCH, p<0.05), while increasing staining intensity (39±5.1% above Con VCH, p<0.05) for cyp 2a5/6, compared to control. Although not significant, there was a downward trend in staining intensity for mEH (20±10.3% of Con VCH, 32±19.3% of Con VCD compared to control). mRNA expression of several bio transformation enzymes were investigated in ovarian interstitial cells. Their expression was affected by repeated dosing with VCH/VCD. Thus, these cells may play an important role in the ovarian metabolism of xenobiotics. (ES08979, ES06694, ES07019).

EFFECT OF BLOCKADE OF GLUTATHIONE (GSH) SYNTHESIS ON OVARIAN FOLLICULAR ATRESIA.

U. Ludferer, Center for Occupational and Environmental Health, University of California, Irvine, Ca.

The mammalian ovary contains a finite complement of oocytes, most of which are destined to undergo the apoptotic process of degeneration termed follicular atresia. Our previous work has shown that healthy, growing follicles, but not atretic follicles, express high levels of the regulatory subunit of glutamate cysteine ligase (GCL), the rate limiting enzyme in the synthesis of the antioxidant tripeptide GSH (Ludferer et al., 2000, Toxicol Sciences,54:257). Depletion of cellular GSH causes apoptosis in many cell types; however, the effect of GSH depletion on ovarian follicular atresia has not been investigated. To test the hypothesis that GSH depletion causes increased follicular atresia, cycling adult rats were treated with ip. injections of 5mmol/kg buthionine sulfoximine (BSO), a specific inhibitor of GCL, in saline or with saline alone at 0700 and 1900h on proestrus (n=2, saline; n=3, BSO) or estrus (n=3 saline; n=4, BSO), and sacrificed at 0700h the next day. One ovary was harvested for total GSH using the Tietze method. The other ovary was cryosectioned and subjected to terminal deoxynucleotidyl transferase-mediated DUTP nick end labeling (TUNEL) using the Cell Death Detection Kit (Roche 372 SOT 2002 Annual Meeting
Molecular Biochemicals, Mannheim, Germany) to detect apoptotic cells and counterstained with hematoxylin. Follicles were also scored for atresia using histological criteria. Logistic regression analysis was used to examine the effects of BSO treatment on atresia while adjusting for estrous cycle stage and follicle type. Ovarian GSH concentrations in BSO-treated animals were 50% lower than in saline treated animals. BSO treatment was associated with a statistically significant increase in follicular atresia as determined by histological criteria and a non-significant increase in follicles containing apoptotic cells by TUNEL. Estrous cycle stage and follicle type were also significantly associated with atresia, with antral follicles from metestrus ovaries most likely to be atretic. These results suggest that ovarian GSH plays a role in preventing follicular atresia.

1824

COMPARISON OF THE EFFECTS OF MTV-1E AND MTV-3 INCREASE ON GROWTH INHIBITION AND CHEMOTHERAPEUTIC DRUG RESISTANCE IN THE PROSTATE CANCER CELL LINE PC-3.

S. H. Garret, R. Dutra, D. A. Soni, S. Somji and M. A. Sosa. Urology, West Virginia University, Morgantown, WV, and Pathology, West Virginia University, Morgantown, WV.

The third isoform of metallothionein (MT-3) has been shown to be overexpressed in prostate cancer and PIN lesions. The expression of MT-3 is highly variable but appears to correlate to the type and grade of the tumor. The expression of MT-3 in prostate cancer was modeled by stably transfecting the commonly utilized prostate tumor cell line, PC-3 with MT-3. Previously it was shown that this caused an inhibition of cell growth and an increased resistance to the chemotherapeutic drugs, vinblastin, etoposide, mitomycin C, cisplatin, and paclitaxel as assayed by the colony formation assay. The PC-3 cell line was also stably transfected with another common expressed MT isoform in the prostate that is not expressed in this cell line, MTV-1E, to see if the above effects are specific to MT-3 or a general feature of all metallothioneins. Five MTV-1E expressing clones were picked for further analysis and the level of the MT-1E protein was found to be 2-3 fold over control (0.9±0.1 to 3.8±0.16 ng MT/µg protein as assayed by immunoblot analysis. The MTV-1E expressing clones had similar resistance profiles to the above chemotherapy drugs as the MT-3 expressing clones, but did not show any change in the growth rate of the cells. The mean doubling time for the MTV-1E expressing clones was 39.2±3.8 hr compared to 34.6±1.5 hr for the blank vector controls whereas the MTV-3 transfected clones had a mean doubling time of 63.4±2.2 hr. These data indicate that the effect of MT-3 on cell growth is a unique feature of the MT-3 isoform probably facilitated by the unique sequence in the beta domain of the protein whereas the effect on the resistance to chemotherapy drugs is a common characteristic of the MT-3 isoform family.

1825

TIME-COURSE ALTERATIONS IN CDK FAMILY GENES IN GERM CELL APOTOPSIS INDUCED BY 2-BROMOPROPANE (2-BP) IN RAT.


Epidemiological surveys and animal experimental studies suggested that exposure to 2-BP could develop reproductive and hematopoietic disorders both in human and experimental animals. In our previous studies, we confirmed that 2-BP exposure results in apoptotic death of testicular germ cells and this process involves the Bcl-2 family genes and the Fas signaling system. In this study, we further explored the roles of cyclin dependent kinase (CDK) family genes in the development of male germ cell degeneration. Sixty-four rats were divided into eight groups, and passively immunized with a dose of 1350 mg/kg of 2-BP for 1-5 days. The rats were killed at 6 h after 1, 2, 3, 5 doses, and 2 or 7 days after final treatments. Histological examination, TUNEL staining of DNA fragments, and Western blotting analysis of CDK family proteins (Cyclin E, CDK 2, CDK 4, Cyclin D1), and the expression of proliferation cell nuclear antigen (PCNA) were conducted. The results show that the expression of PCNA, a marker of proliferating cells, slightly decreased during 2-BP treatment, and it decreased significantly 9 days after treatment. CDK-2 decreased slightly during 2-BP treatment, and it increased significantly 2 days after treatment, while CDK-4 significantly increased after 2-BP exposure, however, it decreased by the day 9 after exposure. Cyclin D decreased markedly during 2-BP treatment, and it decreased significantly 9 days after treatment. This decrease was preceded by the appearance of p21 in the nuclei of germ cells. These results suggest that cyclin D might be involved in the apoptotic death of spermatogonia, while other CDKs might be involved in the secondary degeneration of spermatocytes.

1826

CHARACTERIZATION OF A RAT IN VITRO OVARIAN CULTURE SYSTEM TO STUDY THE OVARIAN TOXICANT 4-VINYLCYCLOHEXENE DIEPOXIDE.

P. I. Devece, L. G. Sigee, and P. B. Hoyer. 1 Physiology, University of Arizona, Tucson, AZ; 2 Pharmacology, University of Arizona, Tucson, AZ; 3 Center for Toxicology, University of Arizona, Tucson, AZ.

The heterogeneity of the mammalian ovary can cause complications for mechanistic studies involving ovarian toxicity. Repeated daily dosing of rats with the occupational chemical 4-vinylcyclohexene diepoxide (VCD) causes depletion of small preantral (primordial and small primary) ovarian follicles. These targeted populations are difficult to study due to their small size and random distribution throughout the ovary. Therefore, a rat ovarian culture system was developed and its susceptibility to VCD was assessed. Ovaries from 4-day old Fisher 344 rats were collected, laid onto membranes floating on defined medium, and covered with a drop of medium. Medium was replaced every two days. Ovaries were thereafter harvested for histologic examination after 15 days (15d). Follicles were classified and counted in every 6th section. Initial follicle populations in ovaries consisted of primordial (80%) and small primary (20%) follicles, whereas somewhat more developed populations were present after 15d of culture (66% primordial, 32% small primary, 2% large primary, few secondary). Rat ovaries in culture for 15d were sensitive to VCD in a concentration-dependent manner. 10 µM VCD had no significant effects (p>0.05), whereas primordial and small primary follicle populations were reduced after culturing 15d with 30 µM VCD relative to controls (by 99% and 80%, respectively; p<0.01). To verify susceptibility of non-ovarian rats to VCD, rats (d4-19 of age) were dosed daily with VCD (80 mg/kg) for 15d. This resulted in a depletion of primordial follicles by 80% (p<0.01) and small primary follicles by 79% (p<0.05). These results demonstrate that ovaries from neonatal rats are sensitive to VCD in culture. Because of the similarity of VCD's targets in vitro and in vivo, this system will be useful for future studies into the mechanism of action of VCD-induced ovarian follicle loss that has been well-characterized in vivo rats (ES06694, ES08979).

1827

NONYLNPHENOL AND ATRAZINE INDUCE INVERSE EFFECTS ON MAMMARY GLAND DEVELOPMENT IN FEMALE RATS EXPOSED IN UTERO.

H. J. Moon, S. Y. Han, C. C. Davis and S. E. Legnon. 1 Department of Toxicology, Korea FDA, NITR, Seoul, South Korea and 2 Reproductive Toxicology Division, NHEERL, USEPA, Durham, NC.

These experiments tested whether exposure to 4-nonylphenol (NP) or atrazine (ATR) during fetal mammary bud outgrowth alters mammary gland (MG) development in adult female offspring. Time pregnant Long Evans rats (N=7-8/group) were treated with NP (10 or 100 mg/kg) or ATR (100 mg/kg), or corn oil on gestation days (GD) 15-19. MGs from fetuses were removed and prepared as whole mounts or histological sections on postnatal days (PND) 4, 22, 33 and 41. Delayed MG development was detected on PND4 in ATR pups, and persisted through PND41 (truncated epithelial progression through the fat pad, fewer primary and lateral branches). High dose NP pups had advanced lobular development of their MG by PND22 (in contrast, controls at PND41). Low dose NP glands were not morphologically different from controls. Immunohistochemical comparisons of mammary sections from PND41 demonstrated low levels of estrogen receptor (ER) staining in control gland stroma and epithelium, and was visibly increased in tissue of pups exposed to NP and ATR. ATR also elevated ER in the stroma surrounding the terminal end buds (TEB). Progestosterone receptor (PR) staining was visibly decreased in the epithelium of NP100 glands vs. control glands. However, PR was present at high levels in epithelium of NP10 glands and was even more prominent in ATR-exposed ductal epithelium and fat cell nuclei. PR was expressed in TEB of all rats, but was undetectable in the TEB cap cell layer. Prolactin staining was elevated in control glands only in glands containing lobule areas (NP-exposed). These qualitative evaluations suggest that the environmental contaminants, NP and ATR, have opposite effects on MG development following gestational exposure. These exposures during a critical period of epithelial outgrowth altered levels of mammary PR and prolactin, which may contribute to the differences in mammary morphology at PND41. (This abstract has been reviewed in accordance with USEPA policy and approved for publication).

1828

ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASES AND AP-1 TRANSCRIPTION FACTOR IN OVOTOXICITY INDUCED BY 4-VINYLCYCLOHEXENE DIEPOXIDE IN RATS.

X. Hu, L. A. Flexa, L. G. Sigee, and P. B. Hoyer. 1 Physiology, University of Arizona, Tucson, AZ; 2 Pharmacology, University of Arizona, Tucson, AZ; 3 Epidemiology/Preventive Medicine, University of Maryland, Baltimore, MD.

Previous studies indicated that ovotoxicity induced in rats by 4-vinylcyclohexene diepoxide (VCD) is rate of acceleration of the normal rate of atresia. Mitogen-activated
protein kinases are thought to play a pivotal role in transmitting transmembrane signals required for cell proliferation or death. This study was designed to investigate whether the MAPK signaling pathways were involved in VCD-induced ovotoxicity. Female F344 rats were dosed daily with VCD (80 mg/kg, i.p.) for 1 day (1D, ovotoxicity has not been initiated), 10 days (10d; early stage reduction. Previous follicular destruction with no follicle loss) or 15 days (15d; significant follicle loss underway). 4 h following the final dose, ovaries and livers were collected. Ovarian small pre-antral follicles (25-100 μm) were isolated, and sub-cellular fractions were prepared. Activities of MAPKs, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal protein kinase (JNK), and p38 kinase, were determined in cytosolic fractions. Activator protein 1 (AP-1) DNA-binding activity was determined in nuclear fractions. The data obtained from ovarian small pre-antral follicles were summarized in the following table (p<0.05; p<0.01 compared with control). VCD did not affect any of these measurements in liver. Taken together, these data support the assertion that ovotoxicity is associated with the activation of JNK and p38 kinases and reduced AP-1 DNA-binding activity after VCD-induced follicle destruction is underway. This kinase activation or inactivation may be crucial in mediating the normal rate of atresia in small pre-antral follicles. (ES09246; ES06694; HD38995).

1829 STRAIN COMPARISON OF ENDOCRINE RESPONSE IN RATS TO BROMODICHLOROMETHANE (BDCM) DURING PREGNANCY.

S. R. Bielmeier, D. S. Best and M. G. Narozny. Curriculium in Toxicology, Univ. of North Carolina Chapel Hill, NC and Reproductive Toxicology Division, NHEERL, USEPA, Research Triangle Park, NC. Sponsor: A. Cummings.

Previously, we reported that BDCM, a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption (FLR) in F344 rats when treated during the luteinizing hormone (LH)-dependent period. We also found that Sprague-Dawley (SD) rats were unaffected at even higher doses. Here, we examined the effects of BDCM on serum progesterone and LH levels during pregnancy in both strains. BDCM, in 10% emulsifier, was dosed by gavage on gestational days (GD) 6-10 (plug day) to GD 0 at 75 mg/kg/day in the F344 rat, and 100 or 200 mg/kg/day in the SD rat. Blood samples were collected from the lateral tail vein once daily on GD 6-11; serum progesterone and LH were measured using DELFIA®. The rats were allowed to deliver and pups were examined postnatally. FLR was confirmed by staining uterine resorption sites with 10% ammonium sulfide. Unlike the F344 rats, the SD rats maintained their litters and progesterone levels; although, similar to the F344 rats, they did show decreased LH levels. F344 rats had an 88% (7/8) incidence of FLR at 75 mg/kg/day, whereas SD rats had 0% (0/10, 0/9) inclusions at both 100 and 200 mg/kg/day. BDCM-treated F344 dams with FLR had reduced progesterone and LH levels on GD 10; mean ± SE LH levels were 0.07 ± 0.04 vs. 0.15 ± 0.04 mIU/ml, respectively. Serum progesterone at 200 mg/kg/day did not show a decrease in serum progesterone levels on GD 10; however, serum LH levels were significantly decreased from controls (0.24 ± 0.06 vs. 0.49 ± 0.06 ng/ml). Importantly, control serum LH values were significantly higher in SD than F344 rats (p<0.0001). Thus, these dramatic strain differences in baseline LH levels and susceptibility to BDCM-induced pregnancy loss exemplify the importance of strain as a consideration when assessing reproductive toxicity.

[Funded by the EPA/UNC Toxicology Research Program, Training Agreement CT827206 Curriculum in Toxicology, UNC-Chapel Hill. This abstract does not necessarily reflect EPA policy.]

1830 A STUDY TO EVALUATE THE EFFECTS OF LEPTIN ADMINISTERED SUBCUTANEOUSLY DURING CRITICAL PHASES OF GESTATION IN MICE.


Leptin is a protein hormone, produced by adipose tissue, involved in the regulation of body fat. Recent results in the literature indicate that leptin administered to normal animals results in rapid loss of body weight primarily due to body fat reduction. Previous studies with Leptin administration continuing into late gestation resulted in reduced postnatal pup survival. The objective of this study was to evaluate if the effects of Leptin previously noted are related to a critical phase of fetal development. For this study, Leptin, 10 mg/kg/day, was administered daily on Gestation Days (GD) 6-15, 15-18, or 18-21. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. Laparohysterectomies were done on GD 18 for one subset of females from each treatment regimen. Another subset was allowed to deliver and rear the offspring to Lactation Day 21. Increased incidences of prolonged gestation and/or dystocia and increase in length of gestation were noted in the groups that were administered Leptin GD 6-15 or 15-18. Body weight reductions typical of Leptin administration were noted in all Leptin-treated groups. Food consumption was reduced GD 15-18 for females treated during that period. Changes in clinical chemistry parameters were typical of previous studies conducted with Leptin. At laparohysterectomies, no changes in intrauterine growth and survival were noted and there were no treatment-related external malformations or variations observed in the fetuses. Mean live litter size and postnatal pup survival were reduced in the groups that were administered Leptin GD 6-18 or 15-18. Mean pup body weights were reduced in the groups that were administered Leptin GD 6-18. The effects of Leptin administration were considered to be most prevalent when the dams were treated late in gestation (GD 15-18), confounding the process of parturition and resulting in higher mortality rate in the F1 pups and decrements in postnatal growth.

1831 TISSUE- AND STRAIN-SPECIFIC EXPRESSION OF MULTIDRUG RESISTANCE PROTEIN 1 (MRP1) IN INBRED MOUSE STRAINS.

M. Z. Dieter, N. J. Cherington and L. D. Lehman-McKeeman. (Proctor & Gamble, Cincinnati, OH. University of Kansas Medical Center, Kansas City, KS and DuPont Pharmaceuticals, Newark, DE)

MRP1 is a member of the ATP-binding cassette (ABC) superfamily of membrane-spanning transporter proteins. These proteins catalyze the ATP-dependent export of many hydrophobic compounds and products of Phase II metabolism, including steroid hormone conjugates, across cellular membranes, and are implicated in the acquired resistance of tumors to chemotherapy agents. The purpose of this work was to compare constitutive expression levels of MRPI in five commonly used strains of female laboratory mice. MRP1 protein was detected in tissue membranes by Western blot analysis. In all strains of mice examined, the highest expression of MRP1 was observed in the reproductive tract, particularly the ovary and fallopian tubes. Marked strain differences were noted in the uterus as expression was highest in C57BL/6 > CD-1 > B6C3F1 > FVB. Strain differences were also noted in skeletal muscle and kidney, in muscle levels of MRP1 were highest in B6C3F1 > FVB > CD-1 > C57BL/6 > 129sv, whereas, in kidney, MRP1 levels were highest in C57BL/6 > 129sv > CD-1 > B6C3F1 > FVB. Examination of MRP1 mRNA in tissues of B6C3F1 mice using branched DNA methods revealed that mRNA levels are not consistent with protein expression, as mRNA levels in ovary were not markedly different from other tissues. These results suggest that the regulation of MRP1 expression in mice is: (1) highly complex; (2) under the control of several factors that may be functionally polymorphic among mouse strains and (3) may contribute to phenotypic differences in xenobiotic metabolism and distribution. In addition, the abundance of MRP1 in the ovary coupled with its ability to transport conjugates of estrogen and other steroids, suggest an important role for this protein in the reproductive tract and implicates it in mechanisms of reproductive toxicology.

1832 EFFECTS ON THE GONADAL DEVELOPMENT OF MEDAKA (ORIZZAZIS LATIPES) EXPOSED AS EMBRYOS TO BROMODICHLOROMETHANE.

A. Thiyagarajah, S. Parker, C. Genninger, L. E. Teuschler, O. Comerly and W. R. Hartley. Environmental Health Sciences (SL29), Tulane University Health Sciences Center, New Orleans, LA; Medical College of Virginia, Richmond, VA; NCEA, USEPA, Cincinnati, OH and Office of Water, USEPA, Washington, DC. Sponsor: C. Miller.

The purpose of this study is to determine the toxic effects of bromodichloromethane (BDCM) on the developing gonad of medaka. BDCM is one of the trihalomethanes, a disinfection-by-product found in the drinking water with developmental/reproductive toxicity concerns expressed in epidemiological studies. Medaka embryos at early high blastula stage were exposed to 10 or 25 mg/L BDCM for 10 days and transferred to continuous-free embryo rearing medium until hatched. Upon hatching, medaka fry were grown-out in spring water for 6 and 12 months. Two controls (embryo-rearing solution and DMSO carrier) were used. Fish were sampled at 6 and 12-month post-exposure and processed for histological evaluation. The evaluated endpoints, indicative of endocrine disruption or other reproductive toxicities were: hermaphroditism; immature gonads; ectopic gonads; male:female ratio, and other pathology. Hermaphroditism was not observed. There were two cases of ectopic gonads (10 mg/L BDCM and DMSO exposure groups) in the female medaka, but none in male medaka. The male: female ratios were skewed towards males suggesting possible androgenic or anti-estrogenic effects.
1833 
OVEREXPRESSION OF INSULIN-LIKE GROWTH FACTOR (IGF)-1 WITH IGF-1 RECEPTOR SIGNALING AND MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) ACTIVATION IN HUMAN UTERINE LEIOMYOMAS.

H. He, H. X. Nie, A. B. Moore, R. G. Richards and D. Dixon, LEP, NIEHS, Research Triangle Park, NC.

Uterine leiomyomas (fibroids) are benign tumors that are clinically apparent in up to 25-30% of women over 30 years of age in the USA. Although the cause of fibroids remains unknown, research in our laboratory suggests that the cell signaling pathways activated by the IGF-1 peptide may be important in tumor growth during the proliferative phase of the menstrual cycle. IGF-1 can bind with either IGF-1 receptor (IGF-1R) or insulin receptor (IR) and both IGF-1R and IR signaling pathways can ultimately lead to mitogen-activated protein kinase (MAPK) activation and DNA transcription, or phosphatidylinositol 3-kinase activation and cell survival. In this study, we examined leiomyoma and normal myometrial tissue from premenopausal women. IGF-1, IGF-1R, IR, and phospho-p44/42 MAPK were immunolocalized in tissue samples. Activation of the IGF-1R signaling pathway was identified by immunohistochemical staining in both leiomyomas and normal myometrium. Overall, our data indicate that leiomyomas associated with nephropathy are concentrated to a higher degree in the IGF-1 receptor than in the leiomyomas. Such findings suggest that the proximal IGF-1 receptor is a potential target for compounds typically considered nephrotoxicants, such as chlorinated solvents.

1834 
EFFECTS OF POLYVINYL ALCOHOL ADMINISTERED IN THE DIET TO RATS ON FERTILITY, EARLY EMBRYONIC DEVELOPMENT, GROWTH AND DEVELOPMENT.

E. D. Redcliff, C. M. Kells and C. C. DeMerli, 3Huntington Life Sciences, East Millstone, NJ and 4Columbia, West Point, PA.

Polyvinyl Alcohol (PVA) is a water-soluble synthetic polymer prepared from the polymerization of vinyl acetate and the subsequent partial hydrolysis of polyvinyl acetate. PVA is used as a polymer for film coating pharmaceutical and dietary supplement tablets where moisture barrier/protection properties are required. PVA was administered in the diet to rats (26/sex/group) at treatment levels of 0, 2000, 3500 and 5000 mg/kg/day for 2 generations. The study design assessed gonadal function, estrous cycle, mating behavior, conception, gestation, parturition, lactation, weaning, and growth and development of F1 and F2 offspring. Parental rats were treated for 70 days prior to mating, throughout mating, gestation and lactation until sacrifice. Clinical observations, body weights and food consumption were routinely recorded. Dietary concentrations were adjusted for each sex to provide the indicated mg/kg/day PVA levels each week basis except during gestation and lactation. Pups were weighed routinely and weaned at 21 days of age prior to selection for the next generation. Uniformed stool was noted predominately at the 3500 and 5000 mg/kg/day levels in F0 and F1 parental animals. This finding was attributed to the high levels of PVA being fed and frequently excreted in the stools. Slight decreases in the mean body weights of F2 males were noted at 2000 and 5000 mg/kg/day. Feed consumption was elevated at the 3500 and 5000 mg/kg/day treatment levels in both generations but not during either lactation period. These increases generally were observed in a dose-related manner (grams/kg/day), as a result of the large amount of PVA being consumed to maintain the caloric intake necessary for normal growth. There were no effects of PVA on F0 or F1 male or female reproductive performance or pup survival, growth, organ weights, and macroscopic or microscopic observations at dietary concentrations of 2000, 3500 and 5000 mg/kg/day.

1835 
METABOLIZING ENZYMES IN THE RAT EPIDIDYMIS AND EREFFENT DUCTS: POTENTIAL ROLE IN MALE REPRODUCTIVE TOXICITY.

S. B. DeTess, D. E. DeGroot and M. G. Miller, Environmental Toxicology, University of California, Davis, CA.

The presence of metabolizing enzymes in the epididymis and efferent ducts may indicate that these structures are potential toxicological targets via metabolic activation. Previously, we proposed that the epididymis may be toxicologically analogous to the kidney. Our studies have focused on identifying and localizing enzymes involved in nephrototoxic responses, such as cytochrome P450(2E1), soluble epoxide hydrolase, and cysteine conjugate β-lyase (β-lyase). We immunolocalized P450(2E1) in the efferent ducts, and confirmed those findings with Western blotting and enzyme activity assays. Likewise, soluble epoxide hydrolase was localized in the efferent ducts, epididymis, and epididymal sperm. Our most recent studies provide evidence for the presence of β-lyase in the efferent duct system. Using immunofluorescent frozen embedded tissues from untreated rats, β-lyase was immunolocalized in 10µm sections probed with anti-β-lyase (gift of James L. Stevens) and Alexafluor488 conjugated IgG (Molecular Probes). Preliminary fluorescent imaging indicated a clearly visible, diffuse signal in the efferent ducts when compared to kidney controls. The caput, corpus, and cauda epididymis were slightly reactive, while the initial segment was devoid of any antibody reactivity. To confirm these findings, kidney and efferent duct protein samples were separated on a 10% SDS gel, fixed to nitrocellulose, and probed with the same antibody. Preliminary immunoblots data indicated the presence of strongly reacting band (~55kDa) in kidney cytosol and mitochondria. A similar-sized band was present in epididymal and efferent duct cytosolic fractions, as well as in the efferent duct S9 pellet. These bands were not present in similar membranes immunolocalized without antibody. Overall, our data indicated that enzymes associated with nephrotoxicity are concentrated to a higher degree in the efferent ducts than in the epididymis. Such findings suggest that the proximal efferent ducts are a potential target for compounds typically considered nephrotoxicants, such as chlorinated solvents.

1836 
IN-LIFE EFFECTS OF DAILY CHLORIDE EXPOSURE AT ENVIRONMENTAL LEVELS IN F0 RATS AND THEIR NEONATES.

B. C. Daturi, V. E. St. Omer, K. M. Misinga, M. M. Mansour, E. O. Abdalla and F. G. Johnson, 1Biomedical Sciences, Tuskegee University, Tuskegee, AL and 2Pharmacology, University of Health Sciences, Kanazawa, Japan.

This in-life study of FO-rats and their neonates was a part of Replicon II of a multigenerational study to evaluate the effects of technical chloral hydrate, Chloroform, a formerly used pesticide and currently an ATSDR priority-listed hazardous substance, is a bioaccumulative and persistent organochlorine pollutant in the environment. The in-life pregnancy and neonatal effects in humans and other mammals exposed to environmental levels of chloral used in this study are unknown. Synagis-Dawley FO-rats were gavage-fed daily with chloral at 0, 0.125, 0.25 and 1.0 mg/kg body weight. In-life measurements included body weight, feed intake, mating index, gestation length and weight, parturition index, litter size and weight, pup mortality at birth, sex ratio and anogenital distance. Chloral exposure did not affect parental body weight and food consumption, mating index, gestation length and weight, and anogenital indices, litter size and weight, and pup mortality at birth. However, on postnatal day one (PND1) chloride treatment increased (P<0.05) male/female ratio of live pups at the 0.125 mg/kg and anogenital distance of the male neonates at the 0.125 and 0.25 mg/kg dose groups. The data indicated that chloride treatment in this study elicited androgenic effects in vivo and hence, chloral may play a role as an endocrine disruptor. (Supported by MH/PF/ATSDR - #U50/ATU30266008B)

1837 
THE EFFECTS OF TETRATHIOMOLYDBATE (TTM, NSC-714598) AND COPPER SUPPLEMENTATION ON FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN RATS.

A. V. Lubrano, J. D. Smith, S. Reusselle, M. D. Meceica, J. E. Tomaszewski, A. C. Smith and B. S. Levine, 1University of Illinois at Chicago, Chicago, IL 2Pathology Associates, Frederick, MD and 3NCTI, Bethesda, MD.

Based on its unique anti-copper action, TTM is being studied as an anticancerigenic agent and is currently in Phase II clinical trials for cancer therapy. The purpose of the present study was to evaluate the toxicity of TTM and the potential of copper supplementation on the reproductive capability of male and female CD rats. Doses of 0, 1, 4, 12 mg/kg/day (0, 6, 24, 72 mg/m^2/day) and 12 mg/kg/day with copper supplementation (110 mg/kg of diet) were given orally by daily gavage to male rats for at least 59 days and to pregnant female rats for about 30 days, including 29 and 15 days, respectively, of dosing prior to cohabitation. There were no
drug-related toxic effects on the estrous cycle or reproductive indices, or maternal toxicity in any female rats. The effect level (NOEL) for female reproductive toxicity was at least 12 mg/kg/day (72 mg/m²/day). Male rats given 12 mg/kg/day showed significant decreases in body weight gains and food consumption and drug-related anemia developed primarily toward the end of the study. Serum ceruloplasmin levels were drug-dependently decreased in all drug-treated male rats. At 12 mg/kg/day, rats in the copper supplemented dose group had serum ceruloplasmin levels 16 times higher than the non-supplemented dose group. TTM given at doses of 12 mg/kg/day resulted in reduced epididymal weights, sperm counts, and sperm motility as well as sperm morphologic abnormalities including excessive sperm fragmentation and histopathologic changes in testis and epididymis. None of these drug-related effects occurred in the 1 and 4 mg/kg/day dose groups. Copper supplementation prevented the adverse sperm effects produced by 12 mg/kg/day of TTM. Therefore, the NOEL for male reproductive toxicity was 4 mg/kg/day (24 mg/m²/day). (Supported by NCI Contract No. N01-CM-87103)

1838
ENHANCED MITOCHONDRIAL TESTICULAR ANTIOXIDANT CAPACITY IN GOTO-KAKIZAKI (GK) DIABETIC RATS: ROLE OF COENZYME Q.
C. M. Palmiere1, D. L. Santos2, R. Sriva3, A. J. Moreno1 and M. S. Santas1.
1. Department of Neurosciences, University of Oviedo, Oviedo, Spain, 2. Laboratory of Biomedical Research, University of Oviedo, Oviedo, Spain, 3. Department of Biomedical Research, University of Oviedo, Oviedo, Spain.

Since diabetes mellitus is associated with impairment of testicular function, ultimately leading to reduced fertility, this study was conducted to evaluate the existence of a cause-effect relationship between increased oxidative stress in diabetes and reduced mitochondrial antioxidant capacity. The susceptibility to oxidative stress and antioxidant capacity (in terms of glutathione, coenzyme Q and vitamin E content) of testis mitochondrial preparations isolated from Goto-Kakizaki non-insulin dependent diabetic rats and from Wistar control rats, 1 year of age, were evaluated. It was found that Goto-Kakizaki mitochondrial preparations showed a lower susceptibility to lipid peroxidation induced by ADP/Fe2+, as evaluated by oxygen consumption and reactive oxygen species generation. The decreased susceptibility to oxidative stress in diabetic rats was associated with an increase in mitochondrial glutathione and coenzyme Q contents, while vitamin E was not changed. These results demonstrate a higher antioxidant capacity in diabetic GK rats, that we suggest is an adaptive response of testis mitochondria to the increased oxidative damage in diabetes mellitus.

1839
THE ARYL HYDROCARBON RECEPTOR (AhR) MAY REGULATE GROWTH, BUT NOT APOPTOSIS OF ANTRAL OVARIAN FOLLICLES.
J. C. Benefiel1, T. M. Lin1, R. E. Peterson2 and J. A. Flax1.
1 Program in Toxicology, University of Maryland, Baltimore, MD and 2School of Pharmacy, University of Wisconsin, Madison, WI.

The AhR mediates the toxicity of chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin. Although little is known about the physiological role of the AhR, our previous work indicates that the AhR may alter the number of antral follicles in the ovary. Mice deficient in the AhR (AhRKO) have 54% fewer antral follicles than wild-type (WT) mice in postnatal (PN) life. The mechanism by which the AhR influences the numbers of antral follicles is not understood. It is possible that the AhR may regulate the number of antral follicles by increasing the rate of growth of immature follicles to the antral stage or by decreasing the rate of death of antral follicles. Thus, the purpose of this study was to test the hypothesis that follicles from AhRKO mice have decreased follicular growth and/or increased apoptosis compared to WT mice. AhRKO and WT (PN 28-35) mice (both on a C57Bl/6 background) were paired with pregnant mare serum gonadotropin to induce growth of immature follicles to the antral stage. After 48 hours, antral follicles were isolated from each ovary and subjected to measurements of follicle size. Additional follicles were cultured in serum free medium to induce apoptosis and then subjected to 3' end labeling to measure the amount of apoptosis. The results indicate that antral follicles from WT mice were significantly larger than antral follicles from AhRKO mice (WT = 21 ± 10 μm; AhRKO = 182 ± 6 μm; n = 6; p < 0.02). In contrast, antral follicles from WT and AhRKO mice and WT mice contained similar amounts of apoptotic DNA after isolation from the ovary (AhRKO = 64.9 ± 7.4 μg/cell; WT = 751 ± 511 μg/cell; n = 3, p < 0.01). These results suggest that the AhR may regulate the growth, but not apoptosis of antral ovarian follicles. Supported by HD 36955 (JAF) and ES01532 (REP).

1840
EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON SERUM INHIBIN CONCENTRATIONS AND INHIBIN IMMUNOSTAINING DURING FOLLICULAR DEVELOPMENT IN SPARGUE-DAWLEY RATS.
B. K. Peroff1, X. Gao2, K. Ohshima3, E. Shi3, D. S. Son4, K. F. Roby4, K. B. Bowman1, G. Wasanabe1, K. Taya1 and F. P. Terranova1,2,4,5.

Intact and hypophysectomized immature rats were pretreated with TCDD (0 or 32 μg/kg p.o.) and sacrificed after defined intervals of follicular development (0, 12, 24, 48, 72 hours after equine chorionic gonadotropin, eCG). TCDD administration resulted in a decrease of serum inhibin in midgestational ovari an sections were decreased at the time of eCG administration (24 hours post-TCDD) in control rats. This decrease of serum inhibin for was not associated with alterations in serum inhibin concentrations. Serum inhibin was suppressed in comparison to control only at 24 hours post-eCG (48 hours post-TCDD) in intact rats that received TCDD. Hypophysectomized animals exhibited no effect of TCDD on serum inhibin at any timepoint. In summary, TCDD reduced serum concentrations of inhibin after the premature increases in FS and LH suggesting that inhibin is not causally related to the initial elevation of FS following exposure to TCDD in this model.

1841
EFFECTS OF INDOLE-3-CARBINOL AND TAMOXIFEN ON OVULATION AND ITS HORMONAL REGULATION — COMPARISON TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).
X. Gao2, B. K. Peroff1, O. Oluola1, K. K. Bowman1 and F. P. Terranova1,2,4,5.

Immature Sprague-dawley rats received daily doses of indole-3-carbinol (I3C, 0.15g/kg/day), diindolylmethane (DIM, 0.40mg/kg/day), tamoxifen (TAM, 0.5mg/kg/day) or vehicle. Follicular development was induced by equine chorionic gonadotropin (eCG, 3 IU) one day after the initial dose. In a hormone replacement study, human chorionic gonadotropin (hCG, 10 IU i.e., 48 hours post-eCG) was used to mimic a normal proovulatory LH surge following treatment with either I3C or TAM. Blood and ovaries were collected throughout follicular development and the number of ovulated eggs was measured on the morning following normal ovulation (72 hours post-eCG). I3C did not alter ovulation, whereas TAM reduced body weight gain at higher doses after 4 days of dosing. Ovuarian weight gain and ovulation were inhibited by both I3C and TAM in a dose-dependent fashion. During the preovulatory period, I3C and TAM blocked normal LH and FSH surges and suppressed serum P4 profoundly without changing circulating levels of E2. After the time of expected ovulation, serum E2 was increased in rats receiving I3C or tamoxifen, whereas serum P4 was decreased dose dependently. DIM exerted no significant effects on any of the endpoints studied even at the highest dose. hCG restored ovulation weight gain and ovulation in TAM-treated rats. However, hCG partially reversed the blockage of ovulation by I3C, although ovarian weight gain was restored to normal. In summary, I3C seems to block ovulation by mechanism(s) different to TCDD (at both ovarian and hypothalamic levels), whereas TAM appears to alter ovulation primarily via effects at the hypothalamic-pituitary axis.

1842
MULTI-GENERATION REPRODUCTION TOXICITY STUDY OF 3, 3', 4, 4' -TETRACHLOROAZOBENZENE ADMINISTERED TO SPARGUE-DAWLEY RATS BY ORAL GAVAGE.
M. Hought1, G. Wolfe1, R. Patel1, J. Quinone2, B. Drag1, B. Collins1, J. Blake1, M. Vallant and J. Bishop1.
1 NIHES, Research Triangle Park, NC, 2 Thieme Research Corporation, Geithsburg, MD, 3PMI, Frederick, MD and 4RTL Research Triangle Park, NC.

3, 3', 4, 4'-Tetrachloroazobenzene (TCAB) is generated as a by-product during the manufacture of environmental degradation of 3, 4-dichloroaniline and its herbicidal derivatives. TCAB was tested for reproductive toxicity in Sprague-Dawley rats.
using the NTP Reproductive Assay by Continuous Breeding protocol. F3 males and females (20/sex/group) were administered TCAB or corn oilavage at concentrations of 0, 1, 3, and 10 mg/kg/day. Mating pairs were 3 F3, lent/ pair and the 3rd litter was raised to adulthood. F4 weanlings were exposed to TCAB at the same concentrations as their F3 parents and on postnatal day 81. 10, 20 pairs of F4 adults/dose were mated to produce 3 F4, litter/pair. Parental observations included body weight, food consumption, organ weights, and gross and microscopic pathology. Litter observations included number of live pups/litter and live pup weight. F1 parental body weights were decreased minimally during the study. There were significant reductions in the number of live F1 pups/litter in all dose groups. Mean body weight was decreased significantly in F1, parents treated with 10 mg/kg. The number of live F1 pups/litter was significantly reduced at 3 and 10 mg/kg and pup weight was decreased at 10 mg/kg. A crossover mating was conducted with the F2 parents to determine which sex was affected by TCAB treatment. Treated F2 males and females from the control and 10 mg/kg groups were paired with naive males and females for 7 days. The reproductive data for the treated males was comparable to the controls. When treated females were cohabitated with naive males, the number of live males/litter was decreased by 53%. There was no effect on the number of prismatic follicles in the ovaries of F3 females treated with 10 mg/kg TCAB relative to controls suggesting that: in vitro developmental toxicity was responsible for the reductions in litter size.

1843
EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO GENISTEIN ON SPERM QUANTITY AND TESTICULAR GENE EXPRESSION IN MICE.
S. M. Samy1, M. R. Fielden1, K. Chow1, and T. R. Zacharewski, 1. Department of Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI 2. Department of Animal Science, Michigan State University, East Lansing, MI.

The objective of this study was to examine the effects of gestational and lactational exposure to phytoestrogen genistein on sperm quality, in vitro sperm fertilizing ability and testicular gene expression. Pregnant mice were gavaged with 0.1, 0.5, 2.5 or 10 mg/kg genistein in corn oil from gestational day (GID) 12 to postnatal day (PND) 21. Anogenital distance (AGD) of male offspring was recorded on PND 7 and PND 21. On PND 21, 105 and 315, body weight and testis weight were recorded. On PND 105 and 315, sperm count and sperm fertilizing ability of F1 males were assessed in vitro. Testicular gene expression was examined using a custom mouse cDNA microarray representing 2689 cDNAs. Testicular RNA from mice in the 10 mg/kg dose group on PND 21 and 105 was compared to that from corresponding control mice. Testis weight was slightly increased in the 10 mg/kg group on PND 105 but not significantly (p<0.05). AGD was decreased on PND 21 in the 10 mg/kg dose group (p<0.05). No effect on spermatogenic or sperm count was observed. in vitro fertilizing ability was significantly increased on both PND 105 (18.7%, p=0.01) and PND 315 (17.9%, p=0.01) in the 10 mg/kg group, but it was decreased by 8.5% on PND 105 in the 0.5 mg/kg dose group (p<0.05). The percent of fragmented eggs was decreased significantly on PND 105 in the 0.5, 2.5 and 10 mg/kg dose groups (p<0.001) and slightly increased on PND 315 for 10 mg/kg dose group. The percentage of one-cell fertilized eggs was significantly decreased on PND 315 in the 0.5, 2.5 and 10 mg/kg dose groups (p<0.001). Microarray data were analyzed using paired t-tests to identify differentially expressed genes. Five genes were up-regulated on PND 105, but there were no changes on PND 21. This study suggests that exposure to genistein in early development increases sperm fertilizing ability in adult mice.

1844
NITROBENZENE-INDUCED TESTICULAR GERM CELL APOTOSIS IN YOUNG AND ADULT FASL-DEFICIENT (GLD) MICE.
A. Naifeh and L. H. Richburg. College of Pharmacy, The University of Texas at Austin, Austin, TX.

Apoptosis of testicular germ cells (GCs) is essential for functional spermatogenesis. We have previously shown that the Fast/CD95 signaling system is important for triggering GC apoptosis in four-week-old male C57Bl/6J (C57) mice treated with MEHP (mono-2-ethylhexyl phthalate). "15 mg/kg"-induced testicular cell injury. However, in B6SnC3H-Fasl/gld/gld mice, that express a nonfunctional form of Fas Ligand, a profound reduction in MEHP-induced GC apoptosis was observed as compared to C57 mice. To test if Fast-mediated GC apoptosis is a common signaling event after testicular cell injury, we evaluated the testicular cell toxicant, nitrobenzene (NB). Four-week-old C57 and gld male mice were dosed with 250 mg/kg NB (600 mg/kg p.o.) and testis were collected at 0, 16, 20, 24 hours. Apoptosis was quantified by determining the percentage of seminiferous tubules that contain greater than TUNEL positive germ cells. Vehicle (corn oil) treated 4 week-old C57 and gld mice exhibited less than 10% germ cell apoptosis. After NB exposure, both C57 and gld mice showed variable (10-65%) increases in GC apoptosis, with a trend for larger increases in the C57 mice at 16 h, whereas similar increases occurred in the testes of gld mice at the later (20 & 24h) time points. Eight-week-old C57 and gld male mice were dosed with NB (800 mg/kg p.o.) and testes were collected at 0, 3, 6, 12, 24 hours. Vehicle-treated C57 and gld mice both showed ~4% apoptosis. As opposed to the young mice, the levels of GC apoptosis remained at control levels for 3, 6, and 12 hours post NB exposure. However, a large increase in GC apoptosis (C57: 50.36 ± 3.11 %; gld: 66.10 ± 12.18%, mean ± SD) occurred after 24 hour post NB treatment. Taken together, these findings suggest that a Fast-independent signaling mechanism may be responsible for NB-induced germ cell apoptosis in both young and adult C57 and gld mice. (Supported by R01ES09145, NS1’s LSAIR and UT Co-op Society).

1845
EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO GENISTEIN ON OVULATION AND EGG FERTILIZING ABILITY IN MICE.
J. Han1, M. Fielden2, S. M. Samy1, F. M. Saarna3, T. Zacharewski1, and K. Choi1. 1. Animal Science, Michigan State University, East Lansing, MI 2. Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI 3. Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.

Genistin (GEN) is an isoflavonoid phytoestrogen in soybeans. Our previous study demonstrated that gestational and lactational exposure to GEN on ovariolytic activity in female offspring. Pregnant C57/Bl6 mice (F-0) bred with DBA/2 male were gavaged with 0, 0.1, 0.5, 2.5, or 10 mg GEN/kg/day body weight in 0.1 ml corn oil from gestational day 12 to postnatal day (PND) 21. No significant effects on fecundity (P>0.1) or 4- and 21-day pup survival were observed. An increase in litter size in the 0.5 mg treatment group was observed (P<0.05). There were no effects on sex ratio, litter weight, or average birth weight of pups among treatment groups (P>0.05). AGD of the female offspring (F-1) in 2.5 mg and 10 mg treatment groups were lower than those in the control group (P<0.05). In 3-4 week old F-1 females, the number of eggs ovulated was not different among treatment groups. In 8.11 week old females, a trend of decrease in the number of eggs ovulated was observed in the 0.5 mg treatment group (P<0.09). Fertilizing ability of eggs from 3-6 week old females in the 0.1 mg/kg treatment group was significantly lower than that of controls (P<0.05), but no effect on the eggs from the 8.11 week old females was observed. Results from this study suggest that exposure to low dose of GEN during gestation and lactation decreases fertilizing ability of eggs from pre-pubertal F-1 females, while high doses of GEN exposure increase the number of non-viable eggs in the mature F-1 females. (This work is supported, in part, by EPA, R827-420-01-01.)

1846
METHOXYCHLOR MAY DAMAGE THE MURINE OVARY AND VAGINA.
C. Borgers1, L. P. Mayer2, R. B. Hoyer1, and J. A. Evans. 1. Program in Toxicology, University of Maryland, Baltimore, MD. 2. Department of Physiology, University of Arizona, Tucson, AZ. and Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD.

Methoxychlor (MxC), an organochlorine pesticide that is currently used to protect agricultural products from insects. Although this chemical is a suspected reproductive toxicant, its effects on the ovary and vagina are not well understood. Thus, the purpose of this study was to test whether MxC induces ovarian atresia and vaginal keratinization by direct action on the ovary and vagina or by indirect action involving the disruption of hormone levels. To test this hypothesis, adult CD-1 mice were dosed with sesame oil (control) or MxC (8, 16, 32 mg/kg) for 10 or 20 days. Estrous cyclicity and vaginal keratinization were evaluated daily using saline-injected mice. After dosing, ovaries were collected for histological evaluation of follicular atresia and blood was collected for measurement of follicle-stimulating hormone (FSH) levels and estradiol (E2) levels. The results indicate that MxC disrupted estrous cyclicity (controls = 33% abnormal cycles, n=7; 8mg/kg MxC = 56% abnormal cycles, n=6; 16 and 32mg/kg MxC = 60% abnormal cycles, n=10 for each dose) and induced vaginal keratinization (controls = 18.8% of 43 mice with MxC = 71.8% of 43 mice, p<0.05). MxC did not alter hormone levels at any dose. After 10 days of dosing, FSH levels were 4.6 ± 0.8 ng/ml in controls and 3.4 ± 0.3 ng/ml in mice treated with 32mg/kg MxC (p<0.05). E2 levels were 2.9 ± 0.7 pg/ml in controls and 121 ± 14.7 pg/ml in mice treated with 32mg/kg MxC (p<0.05). These data suggest that MxC disrupts estrous cyclicity, induces vaginal keratinization, and causes follicular atresia by a direct action on the ovary and vagina and not by an indirect action involving FSH and E2. Supported by HD 54895, T32-ES07265, and the Women’s Health Research Group at the University of Maryland.

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1847 NITRIC OXIDE DECREASES UTERINE CONTRACTILITY BY BLOCKING THE CLOSURE OF ATP-DEPENDENT POTASSIUM CHANNELS.

T. Clippen, T. J. Miller, M. A. Philbert and R. Loch-Caruso, Environmental Health Sciences, The University of Michigan, Ann Arbor, MI.

Both nitric oxide and ATP-dependent potassium channel activators decrease uterine tone and inhibit spontaneous rat and human myometrial contractions during pregnancy. We tested the hypothesis that nitric oxide hyperpolarizes myometrial cells, cultured from gestation day 20 rats, by opening ATP-dependent potassium channels. Functional activity of ATP-dependent potassium channels was measured by evaluating changes in membrane potential using the bio-oxorol dye DBCAC(5) and confocal microscopy. As indicated by a reduction in fluorescence intensity from pre-treatment, ATP-dependent potassium channel opener pinacidil (1 μM) hyperpolarized myometrial cells and increased significantly their membrane potential compared with solvent controls. Gibellinamide (5 μM), an ATP-dependent potassium channel blocker, caused a significant depolarization, indicated by increased fluorescence, followed by hyperpolarization. Pretreatment with the nitric oxide donating compound spermine NONOate (25 μM) inhibited the observed depolarization and augmented the hyperpolarization state caused by glibenclamide. CarboxyPTIO, a nitric oxide scavenger, prevented the inhibitory effects of spermine NONOate on the glibenclamide-induced hyperpolarization. A role for ATP-dependent potassium channels in nitric oxide's effects on contraction frequency is supported by the observation that treatment of uterine strips with glibenclamide blocked relaxation by spermine NONOate. Previous findings support an inhibitory function for oxidative stress. Collectively, these data suggest an equilibrium between oxidative stress and nitric oxide in the maintenance of potassium channels and uterine tone.

1848 HUMAN TISSUE MODEL FOR VAGINAL IRRITATION STUDIES.

M. Klaunzer1, J. Kubilus1, C. L. Cannon1, J. E. Sheasgreen1 and E. C. Lomonte2, 1R & D, MacTek Corporation, Ashland, MA and 2Medical Affairs, Johnson & Johnson, Johnson, Personal Products Company, Skillman, NJ.

Recently, a tissue culture based model of the vaginal epithelium has been developed. Normal, human ectocervico-vaginal (ECV) epithelial cells were induced to form a three-dimensional tissue using specially formulated serum free medium. The in vitro tissue reproduces many of the histological, ultrastructural, and protein expression properties of native tissue, including inter-digitations of cells, glycogen production, and estrogen expression. Initial experiments investigated the use of this tissue model and the MTT tissue viability assay for predicting ECV irritation. Vaginal anti-fungal products, contraceptives and lubricants were exposed to the tissue model and the exposure time which causes a 50% reduction in tissue viability (ET-50) was determined. The ET-50s were compared to rabbit vaginal irritation scores for 7 products which are currently marketed or in clinical trials. Using an ET-50 cutoff of 9.0 hrs, the products could be successfully categorized into minimal or mild irritation classes. Based on these results and the problems associated with obtaining and the handling of human vaginal tissue, it is anticipated that the tissue model will be very useful in assisting product development scientists in developing safe, efficacious products.

1849 EFFECTS OF DIETARY ETHINYL ESTRADIOL (EE2) ON THE MAMMARY GLAND OF MALE CD (SPRAGUE-DAOLEY) RATS.

J. R. Latendresse1, C. Weis2, R. R. Newbold3 and K. B. Delelos4, 1NCCTR, Jefferson, AR, 2PAA Jefferson, AR and 3NIEHS, Research Triangle Park, NC.

Previous dose range-finding experiments had indicated that EE2 and the soy phytoestrogen genistein induced hyperplasia in puberal (PND 50) male mammary glands and that this was among the most sensitive indicators of activity of these compounds. The present study was designed as an adjunct to a subsequent multi-generation bioassay to determine if this effect was reproducible, required continuous dosing, and persisted into adulthood. The parental generation received a soy- and alfalfa-free diet containing 0, 2, 10, or 50 ppb EE2 starting 28 days prior to mating. Dosing continued throughout pregnancy and lactation. F1 pups were fed the same diet as their parents until sacrifice. One male from each of 18 litters was sacrificed at PND 50 and another at PND 90. F1 pups were removed from dose at weaning (PND21) and sacrificed according to the same schedule as the F1 pups. Mammary glands were removed, fixed in 10% NBF, and processed for microscopic evaluation. Hyperplasia of the mammary gland in the F1 rats was evident at PND 50 and PND 90. Mosty mammary ducts were affected at PND 50 (control 2/18, 2 ppb 4/18, 10 ppb 6/18, 50 ppb 14/18), while both ducts and alveoli were hyperplastic in dose dependent-manner at PND 90. In the F2 generation, there was still a trend of ductal hyperplasia at PND 50 (control 3/18, 2 ppb 4/18, 10 ppb 5/18, 50 ppb 7/18), but by PND 90 all groups were similar. We conclude that hyperplasia of mammary gland in male rats is a sensitive indicator of estrogenic activity of EE2, and perhaps other estrogens, but appears to require continuous exposure.

1850 EFFECT OF DIETARY ETHINYL ESTRADIOL (EE2) ON SERUM TESTOSTERONE, PROSTATE WEIGHT, AND ESTROGEN RECEPTORS (ER) IN CD (SPRAGUE-DAOLEY) RATS.

B. Blaydes1, C. Bryant1, R. R. Newbold2 and K. B. Delelos1, 1NCCTR, Jefferson, AR and 2NIEHS, Research Triangle Park, NC.

Estrogen treatment of neonatal rodents has been shown to induce permanent effects on the prostate, but the dose levels at which such permanent effects can be elicited have sparked controversy in recent years. Recently, subclinical doses of EE2 have been reported to effect the reproductive tract in male mice (Thayer et al., Human Reprod., 16, 988, 2001). In a dose range-finding study with dietary HF2 in Sprague-Dawley rats, we had found a marginally increased dorsolateral prostate weight in animals sacrificed at PND 50 in the intermediate dose range (5 ppb, approx. 1 μg/kg body weight/day). The results reported here were obtained in a subsequent multigeneration study using doses of 0, 2, 10, and 50 ppb EE2 administered in a soy- and alfalfa-free diet. Testing was begun for the parental generation 28 days prior to mating and was continued throughout pregnancy and lactation. F1 pups were fed the same diet as their parents continuously. One male from each of 18 litters was sacrificed at PND 50 and another at PND 90. F1, pups were removed from dose at weaning (PND21) and sacrificed according to the same schedule as the F1 pups. Serum testosterone levels were significantly depressed in PND 50 animals at the F1 (10 and 50 ppb) and F2 (10 and 50 ppb) generations. However, no significant effects on ventral or dorsolateral prostate weight were observed in F1, or F2 animals at PND 50 or PND 90. Prostates from 12 animals per treatment group were analyzed by Western blot for levels of ERα and ERβ. Subtle treatment-related changes, not greater than 2-fold, were observed in both prostate lobes, with the most pronounced change being a decreased level of ERβ in the PND 90 ventral prostate. These results indicate that while low doses of EE2 have demonstrable effects on serum testosterone and prostatic estrogen receptors in the male Sprague-Dawley rat, the effect on testosterone appears to be transient and prostate weight is not a robust indicator of these effects under the experimental conditions used here.

1851 EFFECTS OF EXPOSURE TO VINCOLOZIN ADMINISTERED IN A SOY- AND ALFALFA-FREE DIET ON MALE AND FEMALE CD (SPRAGUE-DAOLEY) RATS.

C. Weis1, J. R. Latendresse1, T. J. Bucci1, R. R. Newbold2 and K. B. Delelos1, 1NCCTR, Jefferson, AR, 2PAA Jefferson, AR and 3NIEHS, Research Triangle Park, NC.

Developmental exposure of male pups to the fungicide vinclozolin induces a spectrum of effects that parallel those of the androgen receptor antagonist flutamide. There is a discrepancy in the doses at which some of these effects have been observed (Gray et al., Toxicol. Ind. Health, 15: 48, 1999; Helling et al., Reg. Toxicol. Pharmacology, 32: 42, 2000). Our laboratory is conducting a series of studies of endocrine active compounds, including vinclozolin, administered in a soy- and alfalfa-free diet that has low and carefully monitored levels of the phytoestrogens genistin and daidzein. In the present study, the compound was administered at dose levels of 0, 2, 10, 50, 150, 300, and 750 ppm to dams from gestation day 7 through weaning (28 days) in lactation. Groups of 5 (10 litters, standardized to 4 per sex) were continued on the same dose fed as their parents until sacrifice at PND 50. Ingested doses ranged from approximately 0.2 – 85 mg/kg body weight/day Gestational duration, total and live pups per litter, litter sex ratios, and birth weight were not significantly affected, nor were body weight and food intake for dams or pups. Anogenital distance was decreased only in male pups at the highest dose. Preputial separation was delayed, seminal vesicle cauda/gland weight decreased, and female liver weight increased at 150 ppm and above. Atrophy of the seminal vesicles was noted microscopically only at 750 ppm. In the 10 ppm dose group (approximately 1 mg/kg/day), vaginal opening was significantly delayed and absolute dorsolateral prostate and epididymal weights were increased. When analyzed with body weight as a covariate, the epididymal weight was also elevated at 10 ppm and dorsolateral prostate weight showed a significant quadratic dose trend. These latter results are unexpected and require replication. If replicated, further investigation would be required to explain these effects since they are not consistent with the known antiandrogenic action of vinclozolin.
1852 GENETIC SUSCEPTIBILITY TO INHALED POLLUTANTS.

I. Jaspers and T. Gordon. CEMBL, University of North Carolina at Chapel Hill, Chapel Hill, NC and Environmental Medicine, New York University, Utrecht, NY.

A person's susceptibility to inhaled pollutants is invariably multi-factorial, depending on the individual's genes, age, socioeconomic and ethnic background, and many other variables. However, there is increasing interest in understanding the susceptibility or resistance of individuals to environmental agents based on genetic differences. It is important to understand how genetic polymorphisms in the general population affect responses to environmental agents in order to correctly interpret inter-individual differences in toxicological studies and to ultimately improve risk assessment and environmental policies. This symposium will present current technologies and experimental approaches to identify potential genetic loci regulating susceptibility to inhaled pollutants. The symposium will present evidence from human familial inheritance studies and linkage analysis from inbred mouse strains on the susceptibility to environmental lung diseases induced by inhalation of particles or gases. In addition, the presentations will address state-of-the-art technology, such as novel high-throughput single nucleotide polymorphisms (SNP)-based genotyping methods, which could greatly accelerate the identification of potential susceptibility genes. The presenters will demonstrate that inter-individual differences in responses often observed in toxicological studies may be strongly linked to genetic determinants.

1853 HUMAN STUDIES IN GENETIC SUSCEPTIBILITY.

S. R. Kleber, Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.

Inter-individual variation in human responses to air pollutants suggests that some subpopulations are at increased risk to the detrimental effects of pollutants compared to others. Environmental factors such as previous exposure and nutritional status may influence individual susceptibility. Intrinsically, factors that determine susceptibility include age, gender, and pre-existing disease (e.g. asthma), and it is becoming increasingly clear that genetic background also contributes to individual susceptibility. Environmental exposures and genetic factors associated with disease risk interact in a complex fashion that varies from one person to another. The relationships between the genetic background and disease risk and severity is often evaluated through traditional family-based linkage studies and positional cloning techniques. However, it is becoming increasingly clear that case-control studies based on association of disease or disease subphenotypes with candidate genes may have certain advantages over family pedigree studies for complex disease phenotypes. This is based in part on continued development of quantitative analysis and the discovery and availability of simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs). Linkage analyses with genetically standardized animal models also provide a useful tool to identify genetic determinants of host responses to environmental pollutants. Comparative mapping between the human and mouse genomes will yield candidate susceptibility genes that may be tested by association studies in human subjects. The combined human studies and mouse modeling likely will provide important insight to understanding genetic factors that contribute to differential susceptibility to pollutants in human populations. Support: NI 57412, R826724, ES09606, and P30 ES00002.

1854 ANIMAL STUDIES IN GENETIC SUSCEPTIBILITY.

T. Gordon, Environmental Medicine, NYU School of Medicine, Utrecht, NY.

The contribution of genetic background to a biological process may be more easily determined and partitioned from environmental influence in the inbred mouse than in more heterogeneous species such as humans. The controlled standardized inbreeding of mice has led to genetic homogeneity that cannot be found or duplicated in human populations, making the inbred mouse an important source for genetic modeling of human diseases. Linkage relationships of homologous loci from human and mouse indicate that highly significant homology in gene order and chromosomal structure have been maintained since the evolutionary divergence of human and mouse. Therefore, identification of the chromosomal location of a susceptibility gene in the mouse provides the basis for potentially localizing a homologous gene in the human. Recently, web-based tools have greatly enhanced searches for inter-strain homology (http://www.ncbi.nlm.nih.gov/HomoloGene/).

The considerable resources and energy applied to the mapping of the mouse genome as an integral component of the Human Genome Project emphasize the importance of this murine model for human diseases. It is anticipated that, because the numbers of genes in the human and mouse genomes are increasing almost exponentially with the advent of modern molecular genetic techniques, there will be similar increases in homologous association of disease genes in the two species.

1855 FROM MOUSE TO MAN: ACCELERATING DISCOVERY OF GENETIC SUSCEPTIBILITY LOCI FOR HUMAN DISEASE.


The integrated use of a novel SNP-based genotyping method and gene expression analysis using high-density oligonucleotide microarrays has markedly increased the rate at which complex biological processes can be analyzed. Application of these technologies to murine experimental models of human inflammatory disease has enabled genetic susceptibility loci to be experimentally identified. For example, a genetic locus regulating susceptibility to experimental allergic asthma in an experimental murine genetic model was identified using this approach. The identified murine susceptibility genes provide insight into pathways regulating human inflammatory disease susceptibility. We have also developed a SNP database and software that enable chromosomal regions regulating complex traits in mice to be computationally prioritized. Several examples of how the computational prediction program has been used to identify chromosomal regions regulating complex traits in mice will be presented. The correlation of computationally predicted regions and experimentally verified regions will be assessed.

1856 USING THE HUMAN GENOME PROJECT TO UNDERSTAND HUMAN VARIABILITY IN RESPONSE TO POLLUTANTS.

D. W. Nebert, U Cinco Med. Center, Cincinnati, OH.

Some human diseases are predominantly monogenic, but most diseases--including lung toxicity or cancer--are polygenic (the combinatorial result of two, and usually more than a dozen or hundred, genes) and multifactorial (affected by epistasis and environmental factors). A polymorphism denotes the presence of two or more subgroups in any species population. Ford in the 1940s and Harris in 1958 stated that a polymorphism exists when the "...frequency of a...alleles...has a frequency of 0.01...". Polymorphic and rare variants have frequencies of $q < 0.01$ and $q < 0.01$, respectively. Considering Hardy-Weinberg distribution $(p + q = 1; p^2 + 2pq + q^2 = 1)$, $q$ is the sum of the variant allele frequencies. If $q < 0.01$, the percentage of individuals homozygous for an autosomal recessive trait would be $0.0001%$ (1 in 10,000). All genes are polymeric to varying degrees. Single-nucleotide polymorphisms (SNP) consist of a map of 1-2 million SNPs, 85% of which are located within 5 kb of the nearest exon, but only 24 individuals of several ethnic backgrounds have been examined so far. In just the most recent generation of humans on this planet, every site at which mutations are compatible with life has been mutated an average of 240 times. Similar rates of mutation have occurred in every previous Homo generation. Classic neutral theory of population genetics allows us to infer from this rate that, by analyzing 99% of the most ethically diverse 192 haptopyl genomes on this planet, we will asymptotically approach 11 million (9.06.01) SNPs. The number of rare SNPs will of course be much greater. On average, about 4-10 coding SNPs exist per gene, and 50-60% of these are expected to alter the gene's function. This expectation is rapidly being confirmed; there are 1-2 million SNPs (85% of 11 million) in the 5 and 2 flanks plus introns of the +31,000 genes, plus all cSNPs, which will make up 40-80 haplotypes per gene— with wide variation on a gene-by-gene basis. Ultimately, our knowledge of haplotype patterns of probably several hundred genes will be able to predict one's risk of lung toxicity or cancer caused by a particular inhaled pollutant.

1857 MITOCHONDRIAL TOXICITY PRODUCED BY ANTVIRALS: MODELS AND MECHANISMS.

G. Szentirmay and A. Nieminen, Triangle Pharmaceuticals, Durham, NC, and Case Western Reserve University, Cleveland, OH.

The need for new treatments for serious viral infections, especially with HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus), is urgent. Unfortunately, mitochondrial toxicity has impaired the development of nucleoside analogs, the most successful approach to treatment to date. The intracelular location of mitochondria, heritable defects, unique mechanism of replication and susceptibility to effects on both electron transport and the oxidation of fatty acids combine to provide toxicologists with a formidable challenge. Pathophysiologic implications of mitochondrial function include DNA that is unique to mitochondria (mtDNA), and the fact that mutation and inheritance of this special DNA, and regulation of mtDNA, is critical for normal function. Importantly, mitochondria control both the life and death of cells. The biochemistry of mitochondria is particularly dependent upon γ polymers. Therefore chemicals, in particular nucleoside analogs, that inhibit or alter a substrate for this enzyme can produce mitochondrial toxicity. This Symposium will link topics given above with the best understood but unfortunately fatal toxicity produced by the D
enitomer of a pyrimidine nucleoside known as fuluridin. It is a nucleotide analog that can be used to inhibit the proliferation of certain types of viruses. This research is important because it may lead to the development of new antiviral therapies.

1858 PATHOPHYSIOLOGICAL IMPLICATIONS OF MITOCHONDRIAL FUNCTION.

A. Nieman. Case Western Reserve University, Cleveland, OH.

Compared to other organelles of the cell, mitochondria are unique in that they contain their own DNA. mtDNA encodes genes for various subunits of complex I, III, IV, and V and genes for ribosomal proteins and tRNA. mtDNA is maternally inherited so that new sequence variants are transmitted along maternal lines without the benefit of recombination with male mtDNA. Mutations in mtDNA often result in deficiencies in the respiratory chain, causing defects in heart, skeletal muscle, and nervous system function. Toxic chemicals have long been known to disrupt mitochondrial metabolism and lead to cellular injury, but the mechanisms causing the injury are not yet fully understood.

1859 INHIBITION OR PERTURBATION OF MITOCHONDRIA DNA SYNTHESIS BY ANTIVIRAL B-D-DIDEOXYNUCLEOSIDE ANALOGS COULD BE ONE OF THE KEY MECHANISMS RESPONSIBLE FOR THEIR TOXICITY.

Y. Cheng. Yale University, New Haven, CT. Sponsor: G. Szczep.

B-D-dideoxynucleoside analogs are a class of compounds having anti-cancer and anti-viral activity. The major limiting toxicity encountered is manifested in proliferating tissues. Several B-D-dideoxynucleoside analogs were found to have anti-HIV activity and were used in a long-term basis for the treatment of patients with AIDS. At a clinically relevant dose, these compounds exerted toxicity in non-proliferating tissues after a period of time of use. One of the mechanisms responsible for their delayed toxicity in non-proliferating tissue is the depletion of mitochondrial DNA (mtDNA) on long-term treatment. These compounds exert their action after the mtDNA terminal once the treatment is terminated. In contrast, the delayed toxicity of nucleoside analogs such as I2-AC or FIAU, which could be internalized into mtDNA through the action of DNA polymerase γ and affect mtDNA synthesis as well as transcription, is irreversible since mtDNA does not have the mechanism to remove the internal incorporated nucleoside analog. For the treatment of viral diseases, an ideal nucleoside analog only acts on viral DNA synthesis apparatus but not nuclear or mtDNA synthesis of uninfected cells. In the process of looking for such analogs, M-D-NDC was discovered to have potent anti-HBV and HIV activity by several laboratories including mine but does not have potent activity as other anti-HIV B-D-dideoxynucleoside analogs against mtDNA synthesis. The lack of activity is due to the lack of uptake of the active metabolite in mt although the DNA polymerase γ could still utilize L1-S/1dC-TP as a substrate resulting in DNA chain termination. A summary of our work and understanding in terms of the mechanism of those anti-HIV and anti-HBV nucleoside analogs in affecting mtDNA synthesis will be presented.

1860 MITOCHONDRIAL TOXICITY OF FIAU AND OTHER ANTIVIRAL NUCLEOSIDE ANALOGS: MECHANISMS AND METHODS OF EVALUATION.


FIAU is a thymidine nucleoside analog (NA) with antiviral activity against hepatitis B virus (HBV) in vitro and in animal models of infection. Initially, FIAU demonstrated clinical promise for the treatment of chronic HBV infection but in further clinical trials, long term administration of FIAU resulted in fatal multiorgan toxicity with a delayed onset characterized by refractory lactic acidosis. The clinical manifestations were consistent with mitochondrial dysfunction. FIAU was demonstrated to have effects on mitochondrial function in a number of experiments including evaluation of mitochondrial (mt) DNA replication and production of lactic acid in cells treated with FIAU; measurement of incorporation of FIAU into mtDNA; interaction of FIAU-TP with cellular DNA polymerase gamma which replicates mtDNA; and EM examination of mitochondrial morphology in cells treated with FIAU. FIAU was orally administered to woodchucks infected with or chronicled in the clinical trials. Whereas no adverse events were observed in animals that received clinically relevant doses of FIAU for 28 days, animals that received FIAU for 12 weeks died or were euthanized 78 to 111 days after initiation of treatment. Among other toxicities, these animals demonstrated hepatic insufficiency, lactic acidosis, and hepatic steatois in the final stages of FIAU toxicity. These findings were similar to those seen in humans treated orally with FIAU and are consistent with a deleterious effect on mitochondrial function. Some NAs that are used for the treatment of AIDS can cause a delayed toxicity the symptoms of which include myopathy, peripheral neuropathy, deafness, pancreatitis, hepatic cell failure, and lactic acidosis. These symptoms are consistent with mitochondrial dysfunction and might be mediated through interactions of the NA-triphosphate with DNA polymerase gamma. The experience with FIAU underscores the need for a systematic evaluation of the effects of various nucleoside analogs on mitochondrial function.

1861 MECHANISMS OF ANTIVIRAL NUCLEOSIDE ANALOGUE-INDUCED MITOCHONDRIAL TOXICITY.


Chronic treatment with nucleoside analogues (NRTIs) used in treatment of HIV and HBV has been linked to lactic acidosis, hepatitis, myopathy, pancreatitis, peripheral neuropathy and lipodystrophy. A common mechanism of mitochondrial toxicity has been proposed. Mitochondria each contain 2-3 DNA strands (mtDNA) with thirteen genes encoding enzymes in the electron transport chain. mtDNA replication is independent of nuclear replication but requires DNA polymerase 8 (DNA pol 8), an enzyme encoded by nuclear DNA. DNA pol 8 binds and inserts endogenous mitochondrial triphosphates into the growing DNA strand, but also binds many NRTIs. Most NRTIs are chain terminators of mtDNA replication, but some (e.g. fuluridin) are incorporated into mtDNA. Impaired cell function or cell death follows the gradual depletion of mtDNA to between 15 and 20% of normal cell content. In some cases there is a correlation between inhibition of DNA pol 8 and toxicity in vivo. Zalcitabine (ddC), for example, is the most potent inhibitor of DNA pol 8 and also the most potent inducer of peripheral neuropathy. This correlation does not always hold. Both ddC and d4T (stavudine) are more potent inhibitors of DNA pol 8 than AZT (zidovudine), but only AZT has been associated with clinical signs of myopathy, and evidence of mitochondrial damage in muscle cells in experimental studies in rodents. In our own laboratory, we have shown that NRTI inhibition of adenosine precedes depletion of adenosine in mtDNA in vitro, indicating that mitochondrial toxicity may not explain lipodystrophy associated with highly active antiretroviral therapy (HAART) for HIV. This presentation will review the evidence for mitochondrial toxicity as an explanatory mechanism for all adverse side effects of NRTI treatment.

1862 NOVEL MOLECULAR TOXICOLOGICAL ASSAYS FOR ANTIVIRAL AGENTS.


One of the most preferred targets for the development of the drugs viral infections has been the nucleic acid synthetic machinery. Nucleoside analogs are phosphorylated intracellularly to their triphosphates and can either incorporate into viral nucleic acids or inhibit the viral polymerases. For a compound to be considered selec-
The current "generation" of toxicologists is faced with the challenge of ensuring the safety of biotechnologically produced foods and food ingredients. Food holds a position of inviolability in our culture because of the involuntary nature of its consumption and the pleasure with which it fulfills our nutritional needs. This cultural demand for the absolute purity of food is reflected in literal interpretations of the Delaney clause with no room for de minimis considerations. This demand for absolute purity is under assault with seeds of doubt about the integrity and thoroughness of the testing process for safety of biotechnologically produced substances and has given rise to generalized fears, as exemplified by the Precautionary Principle. This workshop examines key concepts for safety evaluation of biotechnologically produced foods and includes a look at some critical lessons gained from safety evaluation of irradiated food, a strikingly similar problem. What is accomplished within the next 24 months, in terms of safety evaluation of biotechnologically produced food, will profoundly affect the nature and abundance of the food supply for the next three generations of the world's population.

The Surgeon General of the US Army initiated preliminary studies on safety of some irradiated foods in 1948, and short-term studies of 54 foods in 1954, followed by long-term multi-generation animal feeding studies of 21 foods. December 1953 President Eisenhower in an address before the United Nations urged international cooperation and acceptance of the "Atoms for Peace Program." This led to the first International Conference on Peaceful Uses of Atomic Energy. Many countries became then involved in studies of irradiated foods' wholesomeness. Finally, in the 80s and 90s irradiated foods became generally recognized as wholesome. Food is important in international trade and its safety requires worldwide acceptance. Open scientific cooperation and exchange of information across the globe. Not only the Health Authorities of each country must accept the results, but also United Nations' WHO and FAO, as well as the Codex Alimentarius, and WTO. We must assure ourselves scientifically that no harmful effects could go undetected, and we must also reassure the public. The word irradiation leads to many misconceptions and distilling suspicion of harmful effect becomes very difficult. When the evaluation of the data shows that the test group is doing better than the control, the test-group is considered normal or showing no detrimental effect. However, if the control group does better than the test-group, the test-group is suspect. Multi-generation studies can multiply the probability for the test-group to become suspect. These variations may be completely unrelated to the diets. Detailed qualitative and quantitative analyses of all the chemical changes in foods when irradiated, and realization that these changes are similar to those normally occurring in foods when stored and processed by conventional methods, as well as feeding concentrated of the radioactive products to animals in smaller studies were important in proving beyond reasonable doubt that irradiated foods are safe and wholesome. In all this, openness and international scientific cooperation is important as food and food processing requires international acceptance.

The safety assessment of protein products of genes introduced into food crops is multifaceted. The most important food safety concern is to assess the allergenic potential of the introduced protein. This includes comparing the amino acid sequence of the introduced protein to data bases of known protein allergens to confirm that the protein is not related to known allergens. The levels of accumulation of the introduced protein in food parts of the plant and assessment of its potential digestibility are key learnings. Known protein allergens tend to be present at abundant levels in the food and are generally resistant to digestion by processes in the GI tract. The mode of action of the introduced protein should be known, e.g. if it is an enzyme, what are its substrates and the catalytic products. The amino acid sequence of the introduced protein needs to be compared to data bases for known toxic/anti-nutrients to confirm the absence of similarity of the introduced protein to known toxicants. Since known protein toxicants are often through immune mechanisms to cause toxicity when ingested, the potential acute toxicity of the introduced protein can be tested by administration by gavage at high dosages to rodents.

The role of compositional analyses and sub-chronic toxicity studies in the evaluation of substantial equivalence of biotech food crops. Extensive compositional analyses including nutrients, anti-nutrients and secondary metabolites are conducted to evaluate the nutritional status of food crops produced through biotechnology. The results of these compositional evaluations are compared with the results obtained for parental control varieties and conventional commercial varieties in order to examine the placement of the biotech food crop within the natural variability seen for an individual biochemical component. This extensive biochemical sampling, although directed towards nutritional parameters, also assesses unexpected effects due to the expression or the insertion of the introduced genes. In further assess the potential for an unintended or unexpected effect, food crops produced through biotechnology may be incorporated into the diets for sub-chronic toxicity studies in rats. Various toxicological endpoints are evaluated in rats including body weight gain, hematology, clinical chemistry, urine chemistry, organ weights at necropsy and, gross and microscopic pathology. The food and feed safety assessment of Roundup Ready® corn (GA21) included both compositional and toxicological studies. Compositional analysis results showed that, except for a few minor differences that are unlikely to be of biological significance, the grain and forage of GA21 corn were comparable to that of the control corn line and to conventional corn (Salah et al., J. Agricultural Food Chem., 2008, 48, 2395-2312). Similar results were obtained for the toxicological endpoints - the magnitude of the significant differences was small and the values were within historical limits. These data taken together demonstrate that Roundup Ready® corn is as safe and nutritious as conventional corn for food and feed use.
1868 THE USE OF ANIMAL FEEDING STUDIES TO CONFIRM THE SAFETY/WHOLENESSNESS OF NEW CROP VARIETIES.

B. Glen, Federation of Animal Science Societies, Bethesda, MD; Sponsor: G. Burdock.

The US regulatory review process for new crop varieties is accomplished jointly by three federal agencies through a science-based, integrated and multi-disciplinary approach. To date, more than 30 crops produced through biotechnology have been approved for commercialization and these have been substantially equivalent to conventional counterparts for nutrient content and known toxicants. For crops approved to date, feeding studies using livestock have not been required as part of the regulatory process. Further, the US Food and Drug Administration considers the consumption of DNA to be "Generally Regarded as Safe." Nevertheless, to reassure the user of these crops that the food/feed is safe, numerous research studies have been undertaken in various farm animal species around the world. Many crop varieties that are herbicide tolerant or insect protected (e.g. corn, soy, canola, cotton) have been fed to chickens, beef and dairy cattle, swine, sheep and fish in universities around the world using commercial feeding conditions. Findings from over 30 independently conducted studies have indicated no differences for nutrient composition, digestibilities, and animal performance when livestock were fed feedstuffs from conventional and biotech crops. Furthermore, independent studies conducted to determine whether transgenic DNA and proteins can be detected in animal products, no transgenic plant-source DNA and proteins have been found in milk, meat, and eggs.

1869 TRANSGENIC MODELS IN REPRODUCTIVE TOXICOLOGY: APPLICATIONS IN MECHANISTIC AND APPLIED RESEARCH.

D. L. Shuey1 and B. D. Abbous2. 1DuPont Pharmaceuticals Co, Newark, DE and 2Reproductive Toxicology Division (MD67), USEPA, Research Triangle Park, NC.

The development of transgenic models has proved to be an invaluable tool for the isolation of key processes in embryo-fetal development and reproduction. Phenotypic characterization of such models have helped to define critical roles for specific genes and molecules in pattern formation, differentiation and function. Only recently have such models been applied to toxicological investigations. This workshop explores the application of transgenic models in mechanistic and applied toxicological research. Comparison of phenotypes resulting from gene inactivation with patterns of toxicity can provide insight regarding the mechanisms through which agents/compounds act. The models can also be used to directly test hypotheses regarding the roles of specific genes and pathways in the toxic response by administering the test agent(s) to knockout animals. Although the workshop will focus on investigations in developmental and reproductive toxicology, these models also have value in drug development and evaluation of pharmacologic action in non-reproductive tissues/organisms. Such an application will be discussed in which a transgenic model was used to evaluate and develop candidate compounds based on specific mechanistic responses. This workshop will present current applications of transgenic models in toxicology and stimulate discussions of future applications in both mechanistic and applied research.

1870 TRANSGENIC MOUSE MODELS FOR GENE-NUTRITION INTERACTIONS DURING EMBRYONIC DEVELOPMENT.

J. M. Salbaum1, R. H. Fintell1 and C. Kappen. 1Center for Human Molecular Genetics, University of Nebraska Medical Center, Omaha, NE and 2Institute for Biosciences and Technology, Texas A&M University System Health Science Center, Houston, TX.

Congenital abnormalities associated with maternal diabetes include neural tube defects, cardiovascular malformations, and caudal regression/sacral agenesis, and are referred to as diabetic embryopathy. We found such defects can be phenocopied in Isl-1 transgenic mice. Isl-1 is essential for differentiation of motor neurons in the spinal cord and proper pancreatic development. This functional spectrum is similar to that of the Hlx89 gene, which is linked to human sacral agenesis (Cuirranno Syndrome). The parallel developmental phenotypes of Hlx89 mutations in humans and Isl-1 misexpression in mice imply that transcription factors from one cellular pathway can cause comparable pathogenesis in the developing embryo. These phenotypes are not a general property of homeobox transcription factors: classical Hox genes induce different phenotypes that may be modulated by nutrition. We have initiated a genome-wide search for genes regulated by Isl-1 to understand the mechanistic aspects how de-regulated Isl-1 expression can cause developmental defects. Using transgenic mice as tools for gene discovery and gene classification, we have identified target genes on mouse chromosome 9. We have now started to analyze regulatory elements in this gene cluster in vivo. Nature and location of these control elements suggest that Isl-1 is one part of a complex regulatory pattern. We submit that de-regulation of Isl-1 expression could be responsible for conditional defects found in human diabetic embryopathy, suggesting that metabolic factors contribute to the control of Isl-1 expression. To test this hypothesis, we have developed a method to screen large genomic regions for the presence of regulatory elements in vivo and in vitro. The identification of such elements will provide information on upstream factors that regulate Isl-1 expression and the influence of metabolic status on gene expression in diabetic embryopathy.

1871 EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAY IN DEVELOPMENTAL TOXICITY: KNOCKOUT MODELS IN MECHANISTIC TOXICOLOGY RESEARCH.

B. D. Abbous and A. R. Buckalew. Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.

Epidermal growth factor (EGF) and related factors regulate proliferation and differentiation of cells in many tissues and represent important signal transduction pathways in embryo-fetal development. The EGF receptor (EGFR) has six ligands (including EGF and transforming growth factor-alpha, TGF-alpha) with tissue-specific expression patterns. This pathway appears to be involved in the induction of cleft palate and hydromcephalus following exposure to 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD), TCDD exposure in utero alters proliferation of palatal and urinary tract epithelial cells and is associated with disrupted expression of EGF, EGF and/or TGF-alpha. Transgenic knockout (KO) mice which do not express EGF, TGF-alpha or both influenced the incidence and severity of teratogenic responses. For cleft palate, the absence of EGF muted the response, suggesting an EGF-mediated effect. In the urinary tract, the absence of EGF increased responsiveness and this may be related to effects of TCDD on the expression of TGF-alpha in ureteric epithelial cells. These transgenic models provide a means to discriminate the sensitivity of target tissues based on the role of specific ligand(s) in mediating the toxic response. Thus, transgenic models can help evaluate multiple components of complex, interactive pathways, such as those for signal transduction, and can provide important insights into mechanisms of toxicity. This abstract does not necessarily reflect EPA policy.

1872 HYPOTHESIS TESTING IN MALE REPRODUCTIVE TOXICOLOGY USING MUTANT MICE.

K. Buskeide1, M. E. Embree1, H. A. Schoenfeld1, R. J. Rasoulpour1 and D. A. Gray2. 1Brown University, Providence, RI and 2Ottawa Regional Cancer Centre, Ottawa, ON, Canada.

Several mutant mouse models have been used to test mechanisms by which male reproductive toxicity triggers germ cell apoptosis. The Fas system is a paracrine pro-apoptotic signaling pathway; in the testis, Fas ligand (Fasl) is expressed by Sertoli cells and induces apoptosis of germ cells expressing Fas receptor. p53 is a transcription factor which modulates the expression of bcl-2 family members, Fas receptor, and other elements of the apopotic machinery. To examine the possible interactions between p53, germ cell toxicity and Fas, double deficient mice were measured in deficient mice exposed to germ cell toxic (radiation) or a Sertoli cell toxicant (mono(2-ethylhexyl)phthalate, MEEP). Double deficient mice lacking both Fasl and p53 were highly resistant to radiation-induced germ cell apoptosis, while Fasl deficient mice were as sensitive as wild type mice to this injury. In contrast, upon exposure to MEEP all of the deficient strains were protected compared to wild type. These results indicate that the apoptotic response to radiation is p53-dependent and Fasl-independent while the apoptotic response to MEHP is at least partially dependent on both Fasl and p53. A second set of experiments evaluated testicular injury in transgenic mice expressing both a normal endogenous ubiquitin and a dominant negative mutant human ubiquitin that prevents poly-ubiquitination and thus frustrates the processing of abnormal and short-lived proteins involved in signal transduction. When these transgenic mice were made cryoprotected, their testes were more sensitive to this heat-induced injury than wild type controls. These results identify the processing of abnormal and short-lived proteins as an important mechanism underlying the response to injury in the testis, and suggest that impaired activation of polyubiquitination-dependent
signal transduction pathways, such as the anti-apoptotic transcription factor NF-kB, could play a role in the increased sensitivity of the mutant ubiquitin mouse testis.

1873 USE OF GENE KNOCKOUTS TO ESTABLISH A MOLECULAR FRAMEWORK FOR TOXICANT-INDUCED OOYOTE APOPTOSIS IN MURINE AND HUMAN OVARIAN.

J. L. Tilly, Vincent Center for Reproductive Biology, Department of OB/GYN, Massachusetts General Hospital/Harvard Medical School, Boston, MA. Sponsor: D. Shuey.

For many years my lab has studied the role of programmed cell death (PCD) or apoptosis in normal and pathological depletion of germ cells from the ovaries (Dev Biol 1999 217:1-13). A powerful approach to understanding how chemicals kill oocytes has been to examine the impact of “knocking out” PCD regulatory genes, including those encoding signaling molecules (acid sphingomyelinase or ASMase), integrators (Bcl-2 family members) and executioners (caspases), on toxicant-induced oocyte apoptosis in mice (Mol Endocrinol 2001 15:845-853). The evolutionary conservation of the findings is then tested by exposing human ovarian tissue grafted in immunodeficient mice to the same insult and evaluating human oocyte responses. Two examples of our work with gene knockouts in female reproductology will be discussed. The first example describes our efforts to elucidate the intra-cellular mechanisms by which the widely-used anticancer drug, doxorubicin, activates oocyte apoptosis. These studies have shown that ASMase-generated ceramide, the pro-apoptotic Bcl-2 family member Bax, and the PCD executioner enzyme caspase-2 are needed for oocytes to die in response to this toxicant (Nature Med. 1997 3:1228-1232; Genes Dev 1998 12:1304-1314; Nature Med. 2000 6:1109-1114). The second example concerns the identification of PCD-related genomic targets for the aromatic hydrocarbon receptor (AhR) in oocytes that are required for the ovoxic effects of polycyclic aromatic hydrocarbons (PAHs). A novel intracellular signaling pathway involving AhR-driven transcription of the gene encoding Bax in oocytes will be discussed in detail, along with results from parallel studies of human oocyte responses to PAH using a human ovarian xenograft model (Nature Genet 2001 28:in press). Collectively, these studies using gene knockout technologies have identified functionally important biochemical and genetic pathways in oocytes that, when perturbed by xenobiotics, trigger female germ cell death via apoptosis (supported by NIH R01-ES08430).

1874 THE USE OF KNOCKOUT MICE TO DISSOCIATE INDUCTION OF THE HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE (MFO) SYSTEM FROM INHIBITION OF THE 5-LOXYGENASE (5-LO) ENZYME.


Leukotrienes (LTs) are biologically active metabolites of arachidonic acid (AA) that function in normal and pathophysiological conditions. The initial step in LT biosynthesis is the enzymatic conversion of AA to LTA4 by 5-LO. Initially developed to understand the role of LTs in inflammatory conditions, we tested 5-LO KO mice in the collagen-induced arthritis assay. Since this assay is most effectively established in DBA/1 inbred mice, we developed a congenic strain of 5-LO KO mice for phenotypic analysis by consecutive backcrossing for 10 generations onto the DBA/1 genetic background. This effort demonstrated that 5-LO deficient mice had reduced inflammatory responses in the collagen-induced arthritis assay. Since LTs have also been implicated in asthma, a variety of anti-LT therapies have been tested for efficacy in treating the disease. We found in preclinical testing that the 5-LO inhibitors zileuton (Abbott), CJ-11, 802 (Pfizer) and ZDD2138 (Zeneca) all induce the hepatic microsomal MFO system of rodents. Since these three agents are structurally dissimilar, we hypothesized that MFO induction by 5-LO inhibitors could be pharmacologically-mediated. To test this hypothesis, male wild-type and 5-LO KO mice were administered either CJ-11, 802 (200 mg/kg/day) or vehicle by oral gavage for 14 consecutive days. At necropsy, various indices of MFO induction (e.g. liver weight, cytochrome P450 content, and cytochrome c reductase) were measured and all were increased similarly in the treated wild-type and 5-LO KO mice compared to corresponding controls. These results indicate that induction of the hepatic microsomal MFO system in rodents by 5-LO inhibitors is not related to altered LT status.
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March 9–13, 2003

Society of Toxicology

1767 Business Center Drive
Suite 302
Reston, VA 20190-5332

Phone: (703) 438-3115
Fax: (703) 438-3113
E-mail: sothq@toxicology.org
Web site: www.toxicology.org